Lipid and condition analyses of West Greenland marine feeding Atlantic salmon (*Salmo salar* L.)

by

Audrey Dean

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

A regime shift in the Northwest Atlantic in the 1990s caused changes to the diet and productivity of marine-feeding Atlantic salmon (*Salmo salar* L.) off the West Greenland coast. Investigations into the implications of the regime shift for the lipid reserves of summer feeding Atlantic salmon have yet to be conducted, although lipid accumulation is likely linked to survival and may be a potential factor driving observed declines in abundance. For this study, Atlantic salmon were captured and sampled at three locations (Sisimiut: 66.94°N, 53.67°W; Nuuk: 64.17°N, 51.73°W; and Qaqortoq: 60.72°N, 46.04°W) along an 875 km section of the West Greenland coast spanning three years (2009-2011), and dorsal muscle lipids were extracted to assess the variability of lipid stores and potential linkages with fish condition, diet, sea surface temperature (SST), and growth.

Lipids varied between one sea-winter and multi-sea-winter fish but not between North American- and European-origin fish. Lipids varied by latitude of origin in one sea-winter fish, with differences existing only between fish captured at Sisimiut and Nuuk and at Sisimiut and Qaqortoq. Correlations between fish condition and lipids were found for North American one sea-winter fish captured in Sisimiut and Qaqortoq in 2009 and Nuuk in all years. Correlations between weight and lipids were found for North American one sea-winter fish in all communities in 2009 and 2010. These findings suggest the importance of local feeding conditions and maturation in accounting for lipid variability and suggest the need for the incorporation of mesenteric fat and liver lipid reserves into future analyses of Atlantic salmon lipid content.

Percent lipids in North American one sea-winter Atlantic salmon increased with increasing week of capture until standard week 40 at which point lipid content began to decline. Percent lipids correlated negatively with SST, $\delta^{13}C$, and growth rate and positively with $\delta^{15}N$. No

correlations were found between percent lipids and short-term diet. Increasing sea surface temperature appears to have a negative impact on lipid accumulation, with more energy being allocated towards growth as temperatures increase. In addition, arrival timing appears to be an important determinant of forage success, which likely plays a large role in lipid accumulation. Finally, proportions of prey, or any available combination of prey items, in the gut did not correlate with percent lipid, suggesting that singular samplings of gut contents do not accurately reflect long-term trends of the effect of diet on fish conditions as represented by percent lipids in dorsal muscle tissue. More detailed repeat measurements of both gut contents and lipids will be needed to clearly establish diet-condition linkages in future research.

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Bradbury (Fisheries and Oceans Canada; region of origin). Preliminary scale aging was completed by Fisheries and Oceans Canada. Gut contents analyses were conducted by Mindy Sweeney (Normandeau Associates, Inc.) and Heather Dixon (Centre for Cold Regions and Water Science, Wilfrid Laurier University). The data from gut contents analysis was provided by Heather Dixon, who also completed all salmon and prey stable isotope analyses. Scale growth analysis was conducted by Ruth Haas-Castro (NOAA's National Marine Fisheries Service).

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Chapter 1: General Introduction

Atlantic salmon life history

Atlantic salmon (*Salmo salar* L.) historically inhabited the North Atlantic Ocean from the Connecticut River, USA, to Ungava Bay, Canada, in North America and in Europe from rivers in Portugal to rivers emptying into the Barents and White Seas in Russia (Mills 1989; Thorstad *et al.* 2011). Both North American and European Atlantic salmon have experienced significant reductions in population size resulting from range restriction and fragmentation, habitat loss, and exploitation (Jonsson & Jonsson 2011; Thorstad *et al.* 2011). As a result, both groups are now the subject of numerous research projects aimed at understanding contributory freshwater or marine causes for the declines in abundance (Finstad *et al.* 2010; Mills *et al.* 2013; Bal *et al.* 2017; Dixon *et al.* 2017). While both anadromous and resident freshwater forms of Atlantic salmon exist (Mills 1989; Thorstad *et al.* 2011), this study focused exclusively on anadromous fish. Anadromous Atlantic salmon mature in freshwater for one to eight years before migrating to sea to feed on energy-rich marine prey species and grow to maturity (Mills 1989; Nøttestad *et al.* 1997; Thorstad *et al.* 2011; Renkawitz *et al.* 2015). Some male parr, termed "precocious parr" may become reproductively active before migrating to sea (Mills 1989; Thorstad *et al.* 2011). Atlantic salmon from North America overwinter and feed offshore in the Labrador Sea (Mills 1989; Dadswell *et al.* 2010). After one winter, some adults (termed 1SW fish) migrate back to their natal rivers from late spring through early autumn to spawn (Mills 1989; Thorstad *et al.* 2011) and as iteroparous fish may repeat the oceanic migration spawning cycle again (Mills 1989; Thorstad *et al.* 2011). North American fish remaining in the ocean (termed multi-sea-winter or MSW fish) will migrate north to the coast of West Greenland to feed for the summer months and will return to the Labrador Sea the following winter (Mills 1989). Atlantic salmon

generally spend one to five years in the marine environment before spawning (Thorstad *et al.* 2011) depending on whether they return as 1SW or MSW fish.

Climate change in the Northwest Atlantic

An abrupt decline in sea level pressure in the Arctic at the end of the 1980s led to the weakening of the Beaufort Gyre in the first half of the 1990s (MERCINA Working Group 2012). The weakening of the gyre resulted in more freshwater from the Arctic Ocean being transported to the Northern Atlantic Ocean (MERCINA Working Group 2012). These oceanographic changes triggered a "regime shift" in the Northwest Atlantic characterized by the introduction of low salinity water into the Northwest Atlantic in the early 1990s followed by a strong increase in salinity in the late 1990s that resulted in a significant decline in phytoplankton, zooplankton, and planktivorous fish (deYoung *et al.* 2004; MERCINA Working Group 2012) and a decline in the productivity of Atlantic salmon (Mills *et al.* 2013) and their preferred prey, capelin (Nakashima 1996; Carscadden *et al.* 2001; Hedeholm *et al.* 2010; Renkawitz *et al.* 2015).

Feeding habits and diet of Atlantic salmon at sea

A number of Atlantic salmon diet studies have been conducted in the Northwest Atlantic (Templeman 1967, 1968; Lear 1972, 1980; Lacroix & Knox 2005; Renkawitz & Sheehan 2011; Sheehan *et al*. 2012a; Renkawitz *et al*. 2015; Dixon *et al.* 2017), with most studies having been conducted prior to the documented oceanic regime shift that occurred in the 1990s (deYoung 2004; MERCINA Working Group 2012). Since the regime shift, many studies of adult Atlantic salmon marine diets have been completed in the Northeast Atlantic (Andreassen *et al.* 2001; Jacobsen & Hansen 2001, Rikardsen *et al.* 2004). In contrast, in the Northwest Atlantic, only a

few studies have been carried out. These have focused on inshore feeding of post-smolt Atlantic salmon or on feeding off the coast of Labrador (Lacroix & Knox 2005; Renkawitz & Sheehan 2011; Sheehan *et al*. 2012a). Since the regime shift, only two studies have focused specifically on the diet of Atlantic salmon in their summer feeding grounds at West Greenland (Renkawitz *et al*. 2015; Dixon *et al.* 2017).

Atlantic salmon are opportunistic generalist feeders, usually consuming one or two types of prey for a period of time (Lear 1972; Jacobsen & Hansen 2001; Dixon *et al.* 2017). Differences in prey selection have been noted by age, with larger salmon consuming more fish than smaller salmon (Jacobsen & Hansen 2001). In addition, the diet and feeding behavior of Atlantic salmon varies seasonally, with fish feeding more in winter than in autumn (Jacobsen & Hansen 2001; Sheehan *et al.* 2012a; Renkawitz *et al.* 2015; Dixon *et al.* 2017).

Historic diet studies found that Atlantic salmon in the Northwest Atlantic typically consumed capelin (*Mallotus villosus*), barracudina (*Paralepis coregonoides*), sand lance (*Ammodytes* spp.), euphausiids, hyperiid amphipods (*Themisto* spp.), lanternfish (*Notoscopelus* spp.), and boreoatlantic armhook squid (*Gonatus fabricii*) (Templeman 1967, 1968; Lear 1972, 1980; Mills 1989). More recent studies have reported that a smaller variety of prey types compose the majority of the diet (Renkawitz & Sheehan 2011; Sheehan *et al.* 2012a; Renkawitz *et al*. 2015; Dixon *et al.* 2017). Renkawitz *et al.* (2015) and Dixon *et al.* (2017) noted that capelin, sand lance, boreoatlantic armhook squid, and hyperiid amphipods of the genus *Themisto* comprised 85% of the diet of Atlantic salmon in their West Greenland summer feeding grounds, suggesting that diet is temporally and spatially variable.

Marine mortality of Atlantic salmon

Given the difficulties involved with conducting research on migratory species such as Atlantic salmon in the ocean, understanding of the factors contributing to Atlantic salmon marine mortality is limited (Mills 1989; Jonsson & Jonsson 2011). Factors known to influence marine mortality include: fishing activity, predation (e.g. seabirds, marine mammals, and large predatory fish), disease, parasites (e.g. sea lice), prey availability, and sea surface temperature during the first year at sea. However, the relative importance of these factors is not well understood as are the life-stage specific linkages to mortality (Mills 1989; Friedland *et al.* 1993; Friedland 1998; Friedland *et al.* 2000; Chase 2003; Friedland *et al.* 2003; Middlemas *et al.* 2003; Montevecchi and Cairns 2003; Jonsson & Jonsson 2011; Mills *et al*. 2013). Efforts to mitigate mortality and increase productivity (i.e. the closure of marine fisheries and stocking of juvenile fish) have had limited to no success in increasing Atlantic salmon abundance (Mills 1989; Chase 2003; Mills *et al.* 2013). For example, abundances have continued to decline in many North American rivers, including the Penobscot River (Maine, USA), the St. John River (New Brunswick, Canada), and a number of rivers in Labrador and Newfoundland, Canada (Parrish *et al.* 1998; Fay *et al.* 2006; DFO 2017). Studies have suggested that climate change may be contributing to marine mortality and low productivity at sea (Todd *et al.* 2008; Friedland & Todd 2012; Mills *et al.* 2013; Renkawitz *et al*. 2015; Dixon *et al.* 2017), but further research is needed to improve understanding of the contributions of climate change and other factors to declining mortality and productivity so as to better mitigate their effects.

Salmon at Sea Sampling Program

To examine questions related to the impacts of varying marine environments on the decline in survival and productivity of Atlantic salmon, the North Atlantic Salmon Conservation Organization's (NASCO) initiated the Salmon at Sea (SALSEA) program. Under the auspices of the SALSEA program, whole fresh fish were purchased from markets in four West Greenland communities: Nuuk, Qaqortoq, Ilulissat, and Sisimiut in 2009, 2010, and 2011 to obtain biological samples such as: whole stomachs, dorsal muscle tissue, fin clips, heart tissue, kidney tissue, and liver tissue to assess the genetics, population status, and possible determinants of noted declines in the abundance and condition of marine-feeding Atlantic salmon (ICES 2012; Mills *et al.* 2013; Minke-Martin *et al.* 2015; Renkawitz *et al.* 2015; Dixon *et al.* 2017). In addition, samplers recorded the sex, fork length (cm), whole weight (kg), and gutted weight (kg) of all fish sampled and collected scale and otolith samples (Sheehan *et al*. 2012b). During the three years of enhanced sampling, a total of 1200 Atlantic salmon were sampled across the four communities (Table 1) (Sheehan *et al.* 2012b). The continent of origin was determined for all fish, and region of origin was determined from the 2011 sampling for North American-origin fish captured at Nuuk and Sisimiut through genetic analysis of fin clips (King *et al.* 2001; Sheehan *et al.* 2010; Sheehan *et al.* 2012b; Bradbury *et al.* 2016; Anderson 2017). Age was determined through the analysis of collected scales (Power 1987; Sheehan *et al.* 2012b). In addition to genetic studies, biological studies to date have used the obtained samples to analyze diet (Renkawitz *et al.* 2015; Dixon *et al.* 2017), thermal habitat use (Minke-Martin *et al.* 2015), and completed stable isotope analyses on the dorsal muscle tissue and scales for all fish (Dixon *et al.* 2015; Renkawitz *et al*. 2015; Dixon *et al.* 2017), but have not examined lipid levels or correlates of lipids with environment.

Lipids

Atlantic salmon require large energy reserves to successfully migrate from their feeding grounds in the marine environment, spawn in their natal streams, and return to their feeding grounds (Jonsson & Jonsson 2011). Reserved energy takes the form of lipids (fat) in the body tissue (Jonsson & Jonsson 2011). A fish with more lipids in the body tissue will be more likely to survive through migration and spawning (Herbinger & Friars 1991; Todd *et al.* 2008; Jonsson & Jonsson 2011). Therefore, lipid content can be used to assess the likelihood that a fish will survive its return migration to spawn and potentially return to sea (Herbinger & Friars 1991; Todd *et al*. 2008). Past studies have analyzed lipid content in juvenile Atlantic salmon in freshwater (Herbinger & Friars 1991; Simpson 1992; Dempson *et al.* 2004; Jonsson & Jonsson 2005) and adult Atlantic salmon in the nearshore environment prior to river entry and in rivers after spawning (Jonsson *et al.* 1991, 1997). Other studies have assessed the influence of climate on salmon lipid content and fish condition (Todd *et al.* 2008) and the impacts of latitude on lipid content in Atlantic salmon in freshwater (Berg *et al.* 2009; Finstad *et al.* 2010). A number of studies have examined factors controlling lipid accumulation on farmed fish (e.g. Aursand *et al.* 1994; Kadri *et al.* 1995, 1996; Katikou *et al.* 2001). To the author's knowledge, this study will be the first to examine the lipid content of Atlantic salmon at West Greenland.

Lipids were extracted using a modified version of the Folch *et al*. (1957) method. Unlike the Bligh and Dyer (1959) method of lipid extraction, the Folch *et al.* (1957) method provides accurate estimates of lipid content when lipids make up more than 2% of the tissue (Iverson *et al*. 2001). In the Folch method, the percentage of lipids in muscle tissue is calculated from the equation:

$$Percent\ lipid = \frac{m_{dry}}{m_{ground}} * (1 - P_{water}) * 100\% * 20,$$

where m_{dry} is the mass of dry lipid at the end of the extraction process, m_{ground} is the initial ground mass of the tissue used in extraction, and P_{water} is the proportion of water in the muscle (calculated as wet tissue mass – dry tissue mass). In this method, lipids are extracted in duplicate in order to ensure accuracy, with differences between duplicates < 3% (Folch *et al.* 1957). The resulting data were then combined with existing biological (e.g., length, weight) and analytical data (e.g., stable isotope values) as described in the following study objectives to address questions related to the association between lipids and endogenous (e.g., growth rate) and exogenous (e.g., temperature, diet) factors known to control lipid accumulation in fish (Sheridan 1994).

Study Objectives

Chapter 2 sought to describe the pattern of variation in lipid content of Atlantic salmon feeding along the West Greenland coast. This was accomplished by assessing lipid differences by sea age and continent and region of origin. Variation was also assessed on a spatial scale to determine if differences in lipid content existed by latitude of capture. Specifically, the chapter used available data to test the following hypotheses: [H1] lipids vary with life-stage, but [H2] not by continent or region of origin; [H3] lipids vary along a latitudinal gradient with fish captured further north having larger lipid reserves than those captured further south, and [H4] lipids vary positively with fish weight and Fulton's condition factor.

Chapter 3 assessed possible mechanisms driving the observed variation in lipid content of North American one sea-winter Atlantic salmon feeding at West Greenland. This was first accomplished by testing correlations between percent lipids, exogenous factors (e.g. environment and diet), and endogenous factors (e.g. size and growth). Significant correlations were used to

identify potentially significant factors responsible for the observed variation in lipid contents as described in Chapter 2. Specifically, the chapter used available data to test the following hypotheses: lipids of Atlantic salmon captured at West Greenland will correlate positively with [H1] week of capture and [H2] sea surface temperature; lipids will depend on diet, varying positively with [H3] trophic position ($\delta^{15}N$) and [H4] increased inshore feeding ($\delta^{13}C$), and [H5] will depend on prey selection and vary with the proportion of prey found in the gut contents. Additionally, lipid reserves will vary with endogenous factors, having positive correlations between [H6] fork length and [H7] negative correlations with growth rate during the second summer at sea.

As an overall objective, this thesis aims to add to the existing body of literature on summer feeding Atlantic salmon at West Greenland. In addition, research presented in this thesis attempts to fill gaps in knowledge regarding the associations between variations in the lipid content of Atlantic salmon and changes in the marine environment, thereby addressing one of the many suggested potential causes of continued declines in multi-sea-winter fish (ICES 2016), i.e. the impact of changing energy pathways on Atlantic salmon feeding in the marine environment (Renkawitz *et al.* 2015; Dixon *et al.* 2017).

Chapter 2: Variation in the lipid content of Atlantic salmon (*Salmo salar* L.) at West Greenland

Introduction

Atlantic salmon (*Salmo salar* L.) require large energy reserves to migrate successfully from marine feeding grounds to spawn in their natal streams and potentially migrate back to sea (Jonsson & Jonsson 2011). Stored energy takes the form of lipids in the body tissue (Jonsson & Jonsson 2011), with fish making significant use of accumulated energy reserves to support long migrations (Magnoni *et al.* 2006). Thus, a fish with more lipids may have a higher probability of survival through migration and spawning (Herbinger and Friars 1991; Todd *et al.* 2008; Jonsson & Jonsson 2011). Lipids have also been linked to migration strategy, with the energetic status of brown trout, *Salmo trutta*, prior to migration thought to be a determinant of migration distance and duration (Boel *et al.* 2014). Further, body lipids can deplete significantly during return migrations (Jobling *et al.* 1998; Kiessling *et al.* 2004), and it has been demonstrated that migrating adult Atlantic salmon exhibit significant reductions in percent lipids between fjord entry and upstream habitats (Jonsson *et al.* 1991, 1997).

Oceanographic changes in the late 1980s and early 1990s caused a regime change in the Northwest Atlantic (deYoung *et al.* 2004; MERCINA Working Group 2012; Buren *et al.* 2014) that resulted in decreased productivity for many fish species, including Atlantic salmon (Mills *et al.* 2013) and capelin (*Mallotus villosus*) (Nakashima 1996; Hedeholm *et al.* 2010), a primary prey of salmon in the Northwest Atlantic (Carscadden *et al.* 2001). The energy density of *M. villosus* decreased significantly during this period, suggesting that Atlantic salmon may need to spend more time and hence energy foraging to meet the energetic demands for growth, migration, and reproduction (Österblom *et al.* 2008; Mills *et al.* 2013; Renkawitz *et al.* 2015).

Recent studies from West Greenland have shown that contemporary, post-regime shift diets rely on a larger proportion of a smaller variety of prey items, with boreoatlantic armhook squid (*Gonatus fabricii*), *M. villosus*, sand lance (*Ammodytes spp.*), and hyperiid amphipods of the genus *Themisto* comprising 85% of the diet at West Greenland (Renkawitz *et al.* 2015; Dixon *et al.* 2017). In comparison, historical information indicated a greater reliance on *M. villosus*, barracudina (*Paralepis coregonoides*), *Ammodytes* spp., euphausiids, amphiopods, and, to a lesser extent, *G. fabricii* (Templeman 1967, 1968; Lear 1972, 1980). Changes in prey availability have been linked to declining condition in migrating Scottish Atlantic salmon and suggest the importance of a broader understanding of the causes and pattern of lipid variation among at sea feeding Atlantic salmon (Todd *et al.* 2008). Additionally, it has been suggested that climate-driven relationships among lipids, body condition and reproductive success, such as disproportionate reductions in lipid content compared to declines in condition, are important considerations for management analyses (Todd *et al.* 2008).

It has been noted in freshwater studies that lipid stores increase along a latitudinal gradient in juvenile Atlantic salmon (Berg *et al.* 2009; Finstad *et al.* 2010). Northern populations of Atlantic salmon adjust their behavior to retain more energy (i.e. retain lipids) to buffer themselves against longer winter conditions (Berg *et al.* 2009; Finstad *et al.* 2010), which suggests environment is linked to population variation in lipid levels. Latitude-based differences in energy reserves have also been demonstrated for *Menidia menidia*, where northern populations require greater stored energy to survive winter (Schultz & Conover 1997). However, Copeman *et al.* (2017) found no differences in lipid content of juvenile gadids sampled at different latitudes, with the exception of Arctic cod (*Boreogadus saida*) having significantly greater lipid reserves than all other species sampled. It is possible that latitudinal differences in

lipid content may carry over to the marine environment given the presence of a mixed stock at West Greenland and expected linkages between migration distance and duration and differences in lipid content between Atlantic salmon more northern rivers and Atlantic salmon from more southern rivers (Dempson *et al.* 2010).

Given the existing evidence for changes in prey density and size (Nakashima 1996; Carscadden *et al.* 2001; Hedeholm *et al.* 2010), changes in Atlantic salmon productivity in the Northwest Atlantic (Chaput 2012; Mills *et al.* 2013), and reductions in the energy density of key prey species such as *M. villosus* (Renkawitz *et al*. 2015), further research is needed to investigate the implications of these changes for Atlantic salmon lipid content and body condition as they may be related to observed declines in MSW fish returning to freshwater (ICES 2016). The current study evaluates the lipid content of Atlantic salmon at West Greenland to assess its variation in time and space. Specifically, we evaluated differences in lipid content among sampling years, sampling communities, sea ages, continents and regions of origin to test several hypotheses: [H1] lipid reserves vary by life-stage, with non-maturing multi-sea-winter (MSW) Atlantic salmon having greater lipid reserves than one sea-winter (1SW) Atlantic salmon; [H2] lipid reserves do not vary by continent or region of origin; [H3] lipid reserves vary spatially along a latitudinal pattern, with larger lipid reserves found in Atlantic salmon sampled further north, and [H4] lipid reserves positively correlate with individual weight and condition as measured by Fulton's condition factor.

Methods

Sample collection and lipid extraction

Atlantic salmon were sampled from 2009 to 2011 at three West Greenland communities (Sisimiut, Nuuk, and Qaqortoq) across 875 km along the West Greenland coast (Fig. 2.1) as part of the North Atlantic Salmon Conversation Organization's (NASCO) Salmon at Sea (SALSEA) Greenland sampling program (ICES 2012). Sampling occurred from August to October during each year of sampling, and all fish were assumed to have been sampled within a day of capture. Fork length (mm), gutted weight (kg), and whole weight measurements (kg) were recorded and a portion of dorsal muscle tissue from each fish was collected and frozen at -20°C for lipid extraction. The adipose fin of each fish was clipped and preserved for genetic determination of continent (King *et al.* 2001; Sheehan *et al.* 2010) and region of origin (Bradbury *et al.* 2016; Anderson 2017), and scales were collected for age determination (Power 1987). Region of origin information was only available for North American fish captured in 2011 at Nuuk and Sisimiut. Fish were assigned to one of twelve regions of origin on the basis of microsatellite genotyping (Bradbury *et al.* 2016). Fulton's condition factor (Fulton 1904) was calculated using the gutted weight of individual fish to eliminate the impact of stomach fullness on weight.

Percent lipid relative to wet weight (Shearer 1994) was determined gravimetrically using a modified version of the Folch *et al.* (1957) procedure. Following Folch *et al.* (1957), a 10 to 20 mg sample of freeze-dried and ground dorsal muscle tissue was extracted by adding 2mL of 2:1 chloroform:methanol and a 1.6mL of a 0.9% potassium chloride salt wash to each sample followed by homogenization of the sample. The solution was centrifuged at 2300 rpm for 5 minutes, and the supernatant was then removed. The extraction process was repeated three times, and the extracted, lipid-containing 2:1 chloroform:methanol solution was then evaporated under nitrogen gas until dry. Once dry, 2mL of 2:1 chloroform:methanol were added, and duplicate 100mL aliquots of the resulting lipid solution were removed and evaporated until dry lipid

remained. The extracted lipids were weighed with a microbalance precise to 1 µg. Percent lipid was calculated by multiplying the proportion of dry lipid by one minus the proportion of water in the muscle tissue times 100. To ensure that extraction efficiency would not be limited by lipid saturation of the solvent and measurement accuracy, a high solvent-to-sample ratio was maintained throughout the procedure (Iverson *et al*. 2001).

Statistical analysis

All statistical tests were performed using RStudio version 1.0.143 (RStudio Inc., Boston, MA). Percent lipid values beyond 1.5 times the interquartile range ± the median were considered outliers and removed (n=38) as they likely indicate that an error was made during the extraction process. All percent lipid values were logit-transformed for statistical analysis to ensure conformance to normality assumptions (Warton & Hui 2011). Given the lack of statistical difference between sexes by fish length and stomach contents weight noted in other studies using the same set of West Greenland Atlantic salmon samples (Renkawitz *et al.* 2015), we verified this finding by testing differences in lipid content by sex and found no differences. MSW fish that were found to have spawned in previous years through scale analysis were excluded from all statistical analyses (n = 33) due to low sample sizes and to avoid complications associated with energy expenditure due to spawning.

Differences between life-stage and continent of origin (H1, H2) and regional differences for North American origin fish were tested using one-way analyses of variance (ANOVAs) followed by Tukey's HSD *post hoc* test adjusted to account for unequal sample sizes (Spjøtvoll & Stoline 1973). Life-stage differences were tested for North American and European fish combined. The significance of latitudinal variation in percent lipid (H3) was tested for in 1SW

North American and European fish independently using linear regression, with residuals assessed for normality and homogeneity of variance following standard statistical practice as outlined in Zar (2010) using the Shapiro-Wilk test. Similarly, the significance of correlations between percent lipids, condition, and weight (H4) were tested using linear regression. Relationships between percent lipids, condition, and weight were only tested for North American 1SW as they represented the only group with a large sample size across communities and years (lowest n = 44).

Results

In total, lipids were extracted from 1029 Atlantic salmon samples. Percent lipid ranged from 9.15 ± 2.84% to 18.43 ± 6.24% in virgin 1SW fish (n = 962), and 8.25 ± 3.81% to 37.05% in virgin MSW fish (n = 67) (Table 2.1; Fig. 2.2).The condition of North American 1SW Atlantic salmon varied from 0.97 ± 0.10 (mean ± standard deviation) to 1.12 ± 0.14 (Table 2.2).

Percent lipid differed significantly between 1SW and virgin MSW fish (ANOVA, $F_{[1, 1000]} = 8.15$, $p = 0.0044$) (Fig. 2.3). However, there were no significant differences in lipid content between continents of origin for both 1SW (ANOVA, $F_{[1, 960]} = 3.48$, $p = 0.062$) and MSW fish (ANOVA, $F_{[1, 38]} = 0.34$, $p = 0.56$) (Fig. 2.3). North American 1SW fish sampled from Nuuk and Sisimiut in 2011 were found to be from 9 regions of origin. Among the 1SW North American origin fish, there were no significant pairwise differences by region of origin when all fish, regardless of sampling community, were tested together using one-way ANOVA followed by Tukey's HSD *post hoc* test (ANOVA, $F_{[8, 204]} = 1.608$, $p = 0.124$). Similarly, there were no significant pairwise differences by region of origin for North American 1SW fish sampled at Nuuk and Sisimiut using Tukey's HSD *post hoc* test following separate one-way ANOVAs

(Tukey's HSD *post hoc* $p > 0.05$; ANOVA, $F_{[8, 132]} = 2.26$, $p = 0.029$ and ANOVA, $F_{[6, 65]} = 0.84$, $p = 0.55$ for Nuuk and Sisimiut, respectively).

Percent lipid differed significantly by sampling community in 1SW fish (ANOVA, $F_{[2, 959]} = 38.89$, $p < 0.001$) but not in MSW fish (ANOVA, $F_{[2, 37]} = 1.04$, $p = 0.36$; Fig. 2.4). Latitudinal patterns in average percent lipid were not evident in either 1SW ($p = 0.33$) or MSW fish ($p = 0.96$), although northern fish from Sisimiut had significantly greater mean percent lipids (3.74%) than the other more southerly communities (Fig. 2.4).

A weak but significant ($p< 0.05$) relationship between individual fish condition factor and percent lipid was found for 1SW North American fish captured in some communities in some years, with fish captured at Nuuk showing the most consistent annual relationship (Fig. 2.5). Similarly, significant relationships between individual fish gutted weight and percent lipid were found for 1SW North American fish captured in all communities in 2009 and 2010 (Fig. 2.6). Relationships between fish weight and lipids were also tested using whole fish weight and whole fish weight excluding the weight of the stomach contents. The relationships remained significant in both instances ($p < 0.05$) but were weaker.

Discussion:

Data presented here for Atlantic salmon captured at West Greenland indicate that percent lipid varies by sea age but not continent or region of origin. No evidence for a latitudinal pattern was detected, and percent lipids generally did not correlate with body condition, with the exception of fish sampled near Nuuk where a weak but significant positive relationship between condition factor and percent lipids was found. Percent lipids correlated with gutted weight in all three communities in two of the three study years.

The differences detected in percent lipid by sea age are likely linked to diet. Trophic position has been shown to increase with sea age in Atlantic salmon (Dempson *et al.* 2010). While Renkawitz *et al.* (2015) found no change in prey composition in the gut contents of Atlantic salmon at West Greenland with age, the diet of larger Atlantic salmon was increasingly dominated by fish consumption (Jacobsen & Hansen 2001). As fish tend to have higher energy density than lower trophic level prey (Renkawitz *et al.* 2015), increased consumption of fish is potentially linked to the observed higher percent lipid values in MSW fish. Higher lipids may also be related to the onset of maturation, with the acquisition of surplus energy being considered necessary for maturation in Atlantic salmon (Thorpe 1986) and MSW fish being more likely as individuals to return to their natal river for spawning than 1SW fish which may defer spawning and remain at sea. Indeed, Kadri *et al.* (1996) reported rapid increases in lipid levels in maturing fish in early winter and the maintenance of higher levels throughout the following spring. Thus, evident differences in lipid content by sea age are likely driven by maturational differences. Additionally, the low sample size of MSW individuals may also drive noted statistical differences in lipid content.

The lack of a significant difference in percent lipid by continent of origin likely results from fish sharing a common summer feeding area at West Greenland. Exposure to the same conditions (i.e. temperature, prey type and abundance) during the summer months likely diminishes any potential continent of origin differences in percent lipid in adult Atlantic salmon at West Greenland. In addition, Friedland and Todd (2012) noted that the weight and length of North American and European Atlantic salmon at West Greenland has not differed significantly in recent years. The similarity in lipid levels by region of origin for North American salmon may also be due to the use of common over-wintering areas (Dadswell *et al.* 2010). However, sample

sizes were low across different regions of origin, which may hamper abilities to detect any meaningful differences that may exist.

While latitudinal trends in pre-winter lipid stores (Berg et al. 2009) and over-winter lipid loss (Finstad et al. 2010) have been noted in juvenile Atlantic salmon and the estuarine fish *Menidia menidia* (Schultz & Conover 1997), similar patterns were not evident in the adult Atlantic salmon feeding along the West Greenland coast, comparable to the lack of significant difference in the lipid content of Pacific gadids at varying latitude (Copeman *et al.* 2017). Residency time or movement along the coast resulting in limited periods of feeding at any single coastal location (i.e., Dadswell *et al.* 2010) may explain the lack of pattern. Although there is some debate over the movement patterns of Atlantic salmon as they leave their overwintering grounds in the Labrador Sea and move towards summer feeding in West Greenland coastal waters (Reddin & Shearer 1987; Dadswell *et al.* 2010), differences in migration timing from the Labrador Sea or in the timing of arrival and/or arrival location at Greenland may also contribute to the observed lack of a latitudinal pattern in percent lipid. Observed latitudinal relationships may further be confounded by covariates such as feeding responses to localized differences in prey availability or photoperiod, which has been noted to impact feeding efficiency and growth in rainbow trout (*Oncorhynchus mykiss*; Taylor *et al*. 2006) and body lipids depending upon energy taken up through feeding (Shearer 1994), with geographical differences in prey choice and seasonal feeding known to occur among coastal-feeding salmonids (e.g., brown trout, *S. trutta*, in Norway (Rikardsen *et al.* 2006) and Atlantic salmon in West Greenland (Dixon *et al.* 2017). For example, while *M. villosus* was the dominant prey of Atlantic salmon at Nuuk and Qaqortoq, *G. fabricii* was the dominant prey type at Sisimiut for 1SW fish given assumed localized increases in abundance (Dixon *et al.* 2017). Given that percent lipid did not differ

between Qaqortoq and Nuuk but differed between Sisimiut and the two other communities, it appears that prey type may have greater implications for lipid reserves in Atlantic salmon than prey size.

Few studies have assessed correlations between lipids and fish condition, with most studies being conducted on farmed fish (Aksnes *et al.* 1986; Kadri *et al.* 1996; Peterson *et al.* 2005). However, our finding of a weak relationship between percent lipid and condition factor differs from the findings of Peterson *et al.* (2005) who noted a linear increase in percent muscle lipids with condition in cage cultured Bay of Fundy Atlantic salmon for fish with a gonadosomatic index of 0.3 or less. Consistent relationships between lipid levels and condition, however, are not universal, with divergent trends in condition factor and muscle lipid levels having been observed in immature fish (Aksnes *et al.* 1986; Kadri *et al.* 1996). Lack of strong correlation between condition factor and muscle lipid levels has similarly been reported by Kadri *et al.* (1995), suggesting that the assumed relationship between lipid content and condition is possibly more indirect (e.g., Kadri *et al.* 1996) and that direct incorporation of other morphometric measurements is required to increase predictive power as has been shown in the case of *Salvelinus alpinus* (Rikardsen & Johansen 2003). Simpson (1992) noted that condition reflects relative weight for a given length and could change independent of lipid levels as a result of an increase in water content or gonad size. Additionally, lipids are allocated to mesenteric fat and the liver in addition to muscle tissue with mesenteric fat being the primary location of lipid storage (Sheridan 1994). Given that only dorsal muscle lipid reserves were assessed in this study, it is possible that the lack of a consistently significant or strong relationship between percent lipid and condition factor in Atlantic salmon at West Greenland was confounded by the

allocation of lipids to the liver and mesenteric fat, with the allocation of lipids to muscle tissue likely differing between individuals.

Energetic tradeoffs between growth and lipid accumulation in Atlantic salmon (Jonsson & Jonsson 2011) may vary as a result of environmental conditions (Metcalfe *et al.* 2006). The increased presence of lower quality capelin at West Greenland (Renkawitz *et al.* 2015; Dixon *et al.* 2017) and the required maintenance of minimum energy reserves to facilitate survival and prevent starvation (Tveiten *et al.* 1996) suggest that fish may have allocated more of the accumulated energy to increasing size rather than improving condition via lipid storage. The consequences of delayed lipid accumulation include likely delayed maturation as a result of failure to meet minimum condition thresholds (i.e. Thorpe *et al.* 1998), increased exposure to natural mortality factors (e.g., predation, starvation overwinter), and possibly poorer overall available lipid reserves when individuals eventually do return to their natal rivers for spawning. Given that salmonids utilize a large portion of their lipid reserves on their return migration (Keissling *et al.* 2004), any reduction in lipid reserves would result in less energy being available for reproduction, which has been reported by Todd *et al.* (2008) in fish at river entry. Thus, lipid levels as reported here may be predictive of the lower size (e.g., Bal *et al.* 2017) and return rates that have been reported in many salmon rivers (e.g., ICES 2016)

On the basis of significant differences in population-specific C:N ratios obtained from stable isotope analysis, Dempson *et al.* (2010) suggested that Atlantic salmon originating from rivers further north may have greater lipid stores due to increased migration distance. While more northerly captured Sisimiut fish recorded higher lipid levels, the mix of origin within the catch suggests that local marine and feeding conditions likely play a greater role in lipid concentration than population-specific origin. The importance of marine experience in lipid

accumulation is highlighted by differences in average lipid content between Sisimiut and the two more southerly sampling communities and associated differences in prey use. For example, Dixon *et al.* (2017) noted that Atlantic salmon captured at Sisimiut had a significantly different diet than those captured at Nuuk and Qaqortoq, relying heavily on *G. fabricii*. It is possible that the lipid stores of Atlantic salmon consuming *G. fabricii* are higher given the high abundance of *G. fabricii* is with increasing latitude along the West Greenland coast (Zumholz & Frandsen 2006). In addition, larger capelin have been noted further north along West Greenland (Hedeholm *et al.* 2010), which may account for latitudinal differences in lipid content. Conversely, temperature has been noted to increase with increasing latitude along the West Greenland coast (Hedeholm *et al.* 2010). Given that sea surface temperatures are likely lower than the optimal temperature for feed conversion for Atlantic salmon (11°C for post-smolts with optimal temperature decreasing with increasing size; Handeland *et al.* 2008), feeding further north would allow Atlantic salmon to occupy waters closer to their thermal optima, allowing for greater accumulation of lipids.

Data from this study has provided the first quantification of variation in the lipid content of Atlantic salmon captured at West Greenland. Differences in lipid content by sea age are likely associated with differences in diet and as a result of differences in maturation (Thorpe 1986; Kadri *et al.* 1996). The lack of significant difference in the lipid content of North American and European origin Atlantic salmon as well as among North American Atlantic salmon from different regions of origin suggests the importance of local feeding conditions (e.g., Dadswell *et al.* 2010) at a common area for determining both condition and lipid storage. Similarly, the lack of a consistent latitudinal pattern appears to be driven by local conditions which could be reinforced by differences in arrival timing along the coast (Rikardsen *et al.* 2006; Dadswell *et al.*

2010; Dixon *et al.* 2017). The lack of significant correlations between condition, weight, and lipid content suggest underlying variation in physiological processes associated with maturation (Simpson 1992) and the importance of lipids allocated to mesenteric fat and the liver (Sheridan 1994). It is possible that Atlantic salmon at West Greenland are, and have been, accumulating less lipids from feeding since the oceanic regime shift (deYoung *et al.* 2004; MERCINA Working Group 2012; Buren *et al.* 2014), which likely has implications for survivorship and is consistent with reported patterns of declining abundance (ICES 2016).

Table 2.1 Average percent lipids (± standard deviation) of 1SW and MSW Atlantic salmon captured at West Greenland by continent of origin, year of capture, and community of capture.

Continent of Origin	Year	Community	1SW n	1SW Lipids (%)	MSW n	MSW Lipids (%)
Europe	2009	Sisimiut	4	17.32 ± 4.41	0	
		Nuuk	10	12.95 ± 4.17	2	20.76 ± 12.28
		Qaqortoq	10	16.17 ± 4.79	2	22.18 ± 7.61
	2010	Sisimiut	11	13.22 ± 7.21	0	
		Nuuk	36	11.73 ± 5.17	2	15.01 ± 10.19
		Qaqortoq	10	12.00 ± 4.37	2	13.51 ± 3.97
	2011	Sisimiut	6	15.63 ± 5.73	2	19.58 ± 8.32
		Nuuk	9	18.43 ± 6.24	1	17.19
		Qaqortoq	10	16.94 ± 8.80	7	8.59 ± 1.32
North America	2009	Sisimiut	67	18.03 ± 5.54	5	11.95 ± 5.10
		Nuuk	172	12.98 ± 5.62	12	11.03 ± 3.27
		Qaqortoq	44	17.54 ± 7.46	8	23.74 ± 3.95
	2010	Sisimiut	70	12.45 ± 5.10	0	
		Nuuk	135	9.15 ± 2.84	4	17.27 ± 8.97
		Qaqortoq	52	12.08 ± 4.38	0	
	2011	Sisimiut	73	17.52 ± 7.03	1	37.05
		Nuuk	141	13.30 ± 6.77	8	8.25 ± 3.81
		Qaqortoq	102	9.18 ± 2.42	11	20.76 ± 8.14

Table 2.2. Biological characteristics of North American 1SW Atlantic salmon sampled from West Greenland for 2009 to 2011. Values reported represent the mean ± standard deviation.

Year	Community	Fork Length (mm)	Gutted Weight (kg)	K
2009	Sisimiut	654.0 ± 30.3	2.96 ± 0.45	1.05 ± 0.08
	Nuuk	651.0 ± 30.1	3.04 ± 0.54	1.09 ± 0.10
	Qaqortoq	657.7 ± 27.5	2.92 ± 0.44	1.05 ± 0.09
2010	Sisimiut	667.0 ± 31.7	3.10 ± 0.52	1.04 ± 0.08
	Nuuk	661.5 ± 33.0	3.27 ± 0.64	1.12 ± 0.14
	Qaqortoq	679.9 ± 41.6	3.22 ± 0.70	1.01 ± 0.11
2011	Sisimiut	654.2 ± 28.3	2.88 ± 0.40	1.03 ± 0.07
	Nuuk	658.1 ± 30.8	3.01 ± 0.56	1.05 ± 0.09
	Qaqortoq	680.8 ± 32.2	2.88 ± 0.50	0.97 ± 0.10

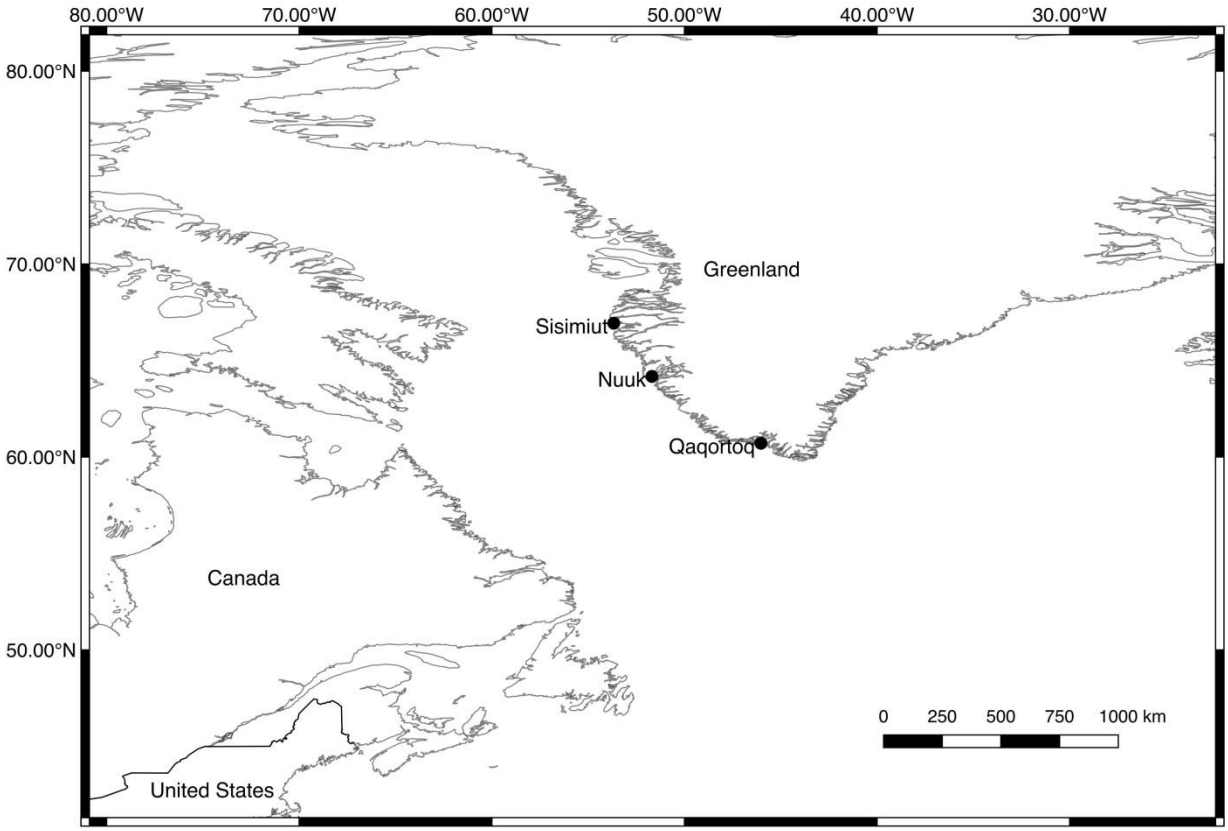

Figure 2.1. Atlantic salmon sampling locations in West Greenland from 2009 to 2011.

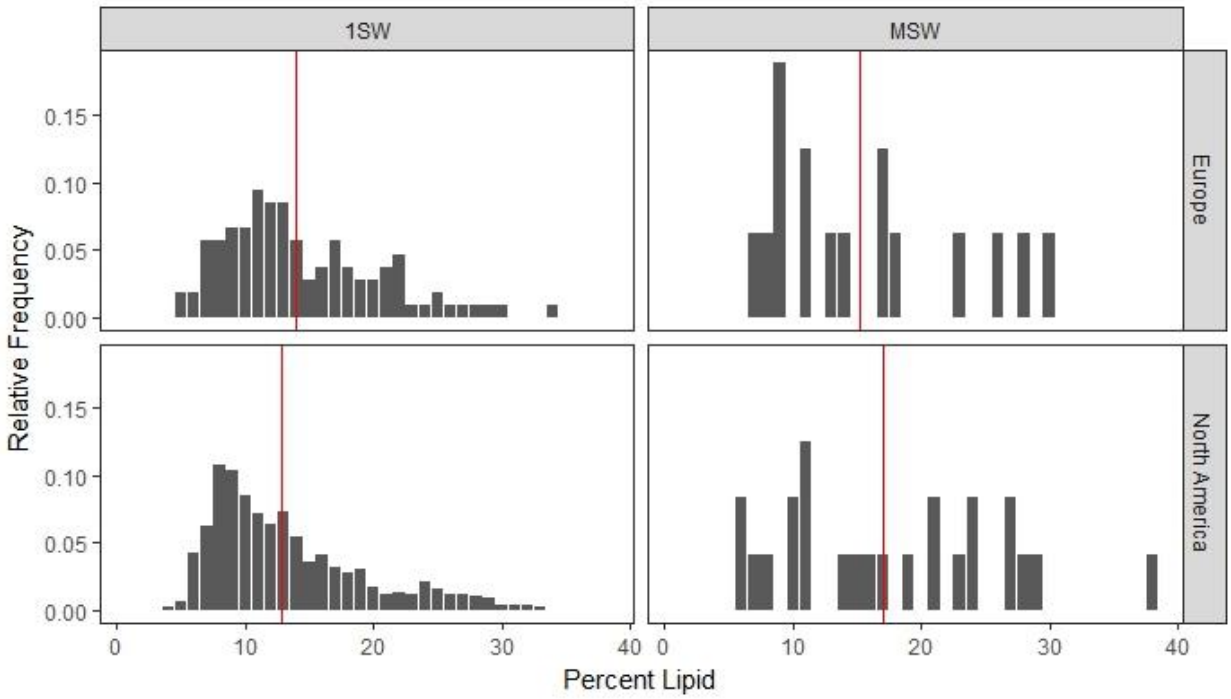

Figure 2.2. Relative frequency of percent lipid in Atlantic salmon captured at West Greenland by total sea age and continent of origin. Vertical lines represent average percent lipid values.

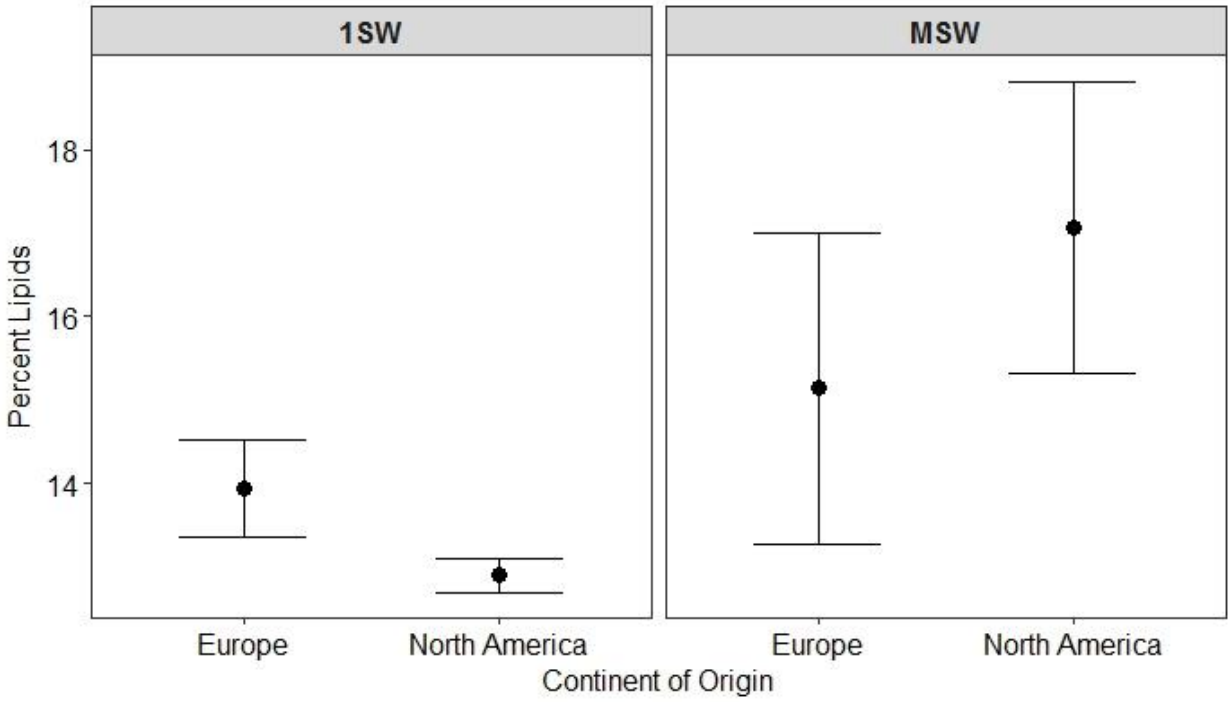

Figure 2.3. Average percent lipid of North American-origin and European origin one sea-winter and multi-sea-winter Atlantic salmon captured at West Greenland by total sea age. Error bars represent standard errors. No significant differences between continents of origin were found for either age-class.

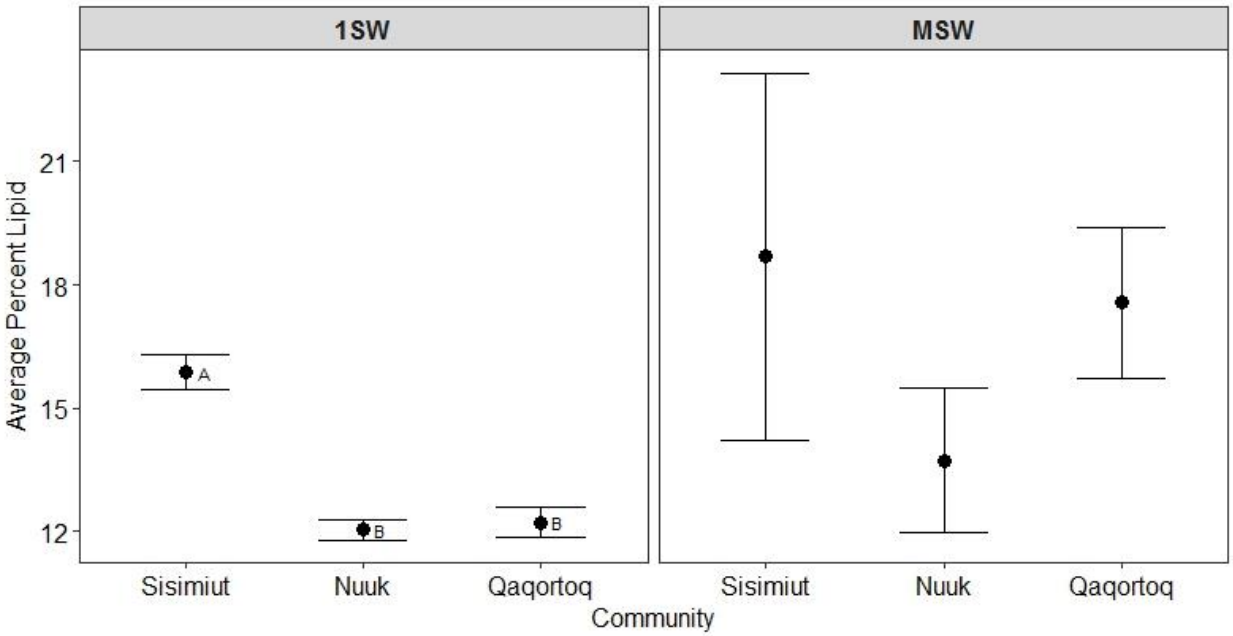

Figure 2.4. Average percent lipid of one sea-winter and multi-sea-winter Atlantic salmon captured at West Greenland by sampling community and total sea age. Communities are in order from furthest north to furthest south. Error bars represent standard errors. When significant differences between communities were found, homogenous groups are indicated with letters.

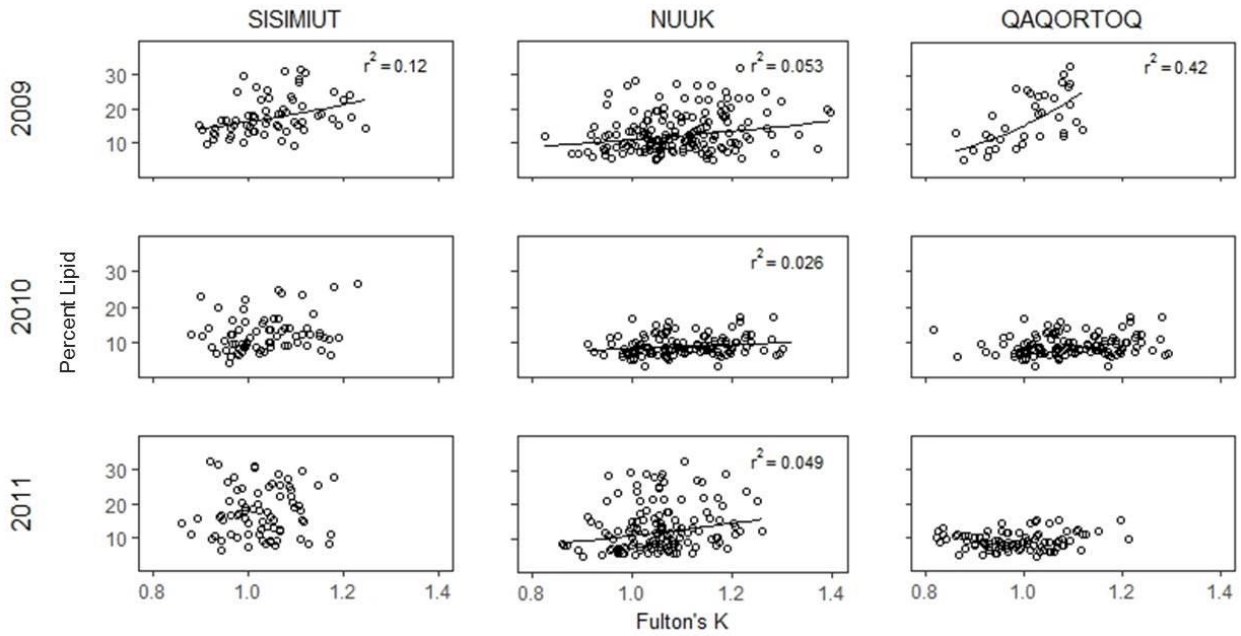

Figure 2.5. Correlation between Fulton's K condition factor and percent lipid for North American-origin 1SW Atlantic salmon captured at West Greenland. R^2 values are reported for significant relationships ($p < 0.05$) plotted as solid lines. Non-normal residuals were present in the regression for Nuuk 2011.

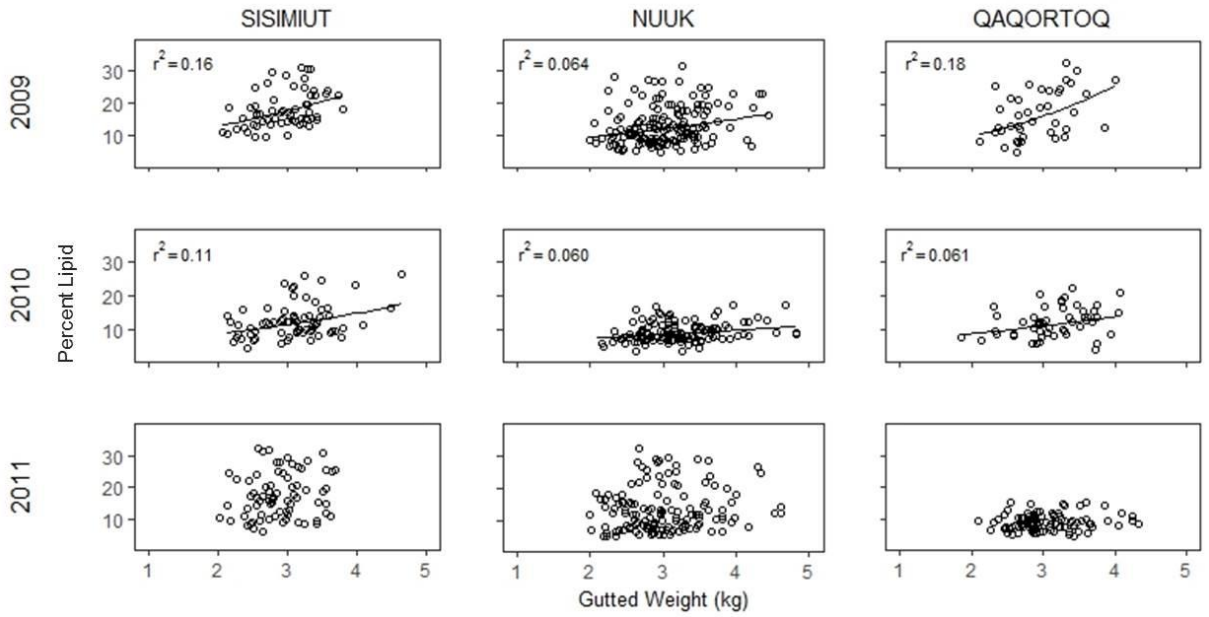

Figure 2.6. Correlation between gutted weight and percent lipid for North American-origin 1SW Atlantic salmon captured at West Greenland. R^2 values are reported for significant relationships ($p < 0.05$) plotted as solid lines. Non-normal residuals were present in the regression for Nuuk 2011.

Chapter 3: The impacts of temperature, diet, and growth on the lipid content of Atlantic salmon (*Salmo salar* L.) captured at West Greenland

Introduction

Atlantic salmon (*Salmo salar* L.) migrate to sea to feed on energy dense prey in the North Atlantic to grow and to accumulate energy reserves in the form of lipids needed for maturation, migration, and reproduction (Thorpe 1986; Nøttestad *et al.* 1999; Magnoni *et al* 2006; Jonsson & Jonsson 2011; Renkawitz *et al.* 2015). Also important for growth are the temperature conditions under which it occurs, with interaction between temperature and energy acquisition playing an important role in the determination of growth rate and body condition (Jonsson & Jonsson 2011). For example, changes in sea surface temperature have been linked to changes in the condition and growth of Scottish, Norwegian, and North American Atlantic salmon, suggesting that changes to prey availability due to ocean warming have negatively influenced survival (Todd *et al.* 2008; Friedland & Todd 2012; Jonsson *et al.* 2016). Temperature and prey further interact through the influence of temperature on food conversion efficiency, with the optimal temperature for food conversion decreasing with size in post-smolts (Handeland *et al.* 2008). Additionally, climatic variability has been linked to the productivity and recruitment of Atlantic salmon at sea, with the impacts of climate driven environmental variability depending on continent of origin (Friedland *et al.* 2014).

An oceanic regime shift in the early 1990s in the Northwest Atlantic triggered a decline in ocean productivity (deYoung *et al.* 2004; MERCINA Working Group 2012) that both affected Atlantic salmon (Mills *et al.* 2013) and its key prey capelin (*Mallotus villosus*; Nakashima 1996; Carscadden *et al.* 2001; Buren *et al.* 2014; Renkawitz *et al.* 2015; Dixon *et al.* 2017). Most notable was the decline in capelin energy density thought to have negatively affected Atlantic

salmon through a lowering of overall energy consumption (Renkawitz *et al*. 2015). Prior to the regime shift, West Greenland Atlantic salmon tended to consume capelin, barracudina (*Paralepis coregonoides*), sand lance (*Ammodytes* spp.), euphausiids, *Themisto* spp., and some boreoatlantic armhook squid (*Gonatus fabricii*) (Templeman 1967, 1968; Lear 1972, 1980). Post-regime shift diet studies have noted dietary shifts by Atlantic salmon at West Greenland, with fish now primarily relying on fewer prey species in a diet where capelin, sand lance, *Themisto* spp., and boreoatlantic armhook squid comprise 85% of the prey items (Renkawitz *et al.* 2015; Dixon *et al.* 2017). By increasing their consumption of potentially less energy-dense prey, Atlantic salmon may now have to expend more time and energy foraging for prey to meet their energetic requirements for growth, migration, and reproduction (Österblom *et al.* 2008; Renkawitz *et al.* 2015).

Lipid reserves in summer-feeding Atlantic salmon at Greenland are known to be highly variable (coefficient of variation = 47.37%; Dean *et al.*, in review), and the variation is likely linked to diet (Dixon *et al.* 2017), maturation (Thorpe 1986; Kadri *et al.* 1996), and thermal experience (Handeland *et al.* 2008). While it has been suggested that the energetic demands of foraging Atlantic salmon have increased with the decline in the quality of capelin (Österblom *et al.* 2008; Renkawitz *et al.* 2015; Dixon *et al.* 2017), Dean *et al.* (in review) suggest that the consumption of boreoatlantic armhook squid may actually have resulted in greater lipid accumulation compared to the consumption of capelin. Boreoatlantic armhook squid are in high abundance between 68°N and 73°N (Zumholz & Frandsen 2006) and compare favourably with capelin in terms of energy density (Elliott & Gaston 2008; Hedeholm *et al.* 2011).

Given the extent and nature of the changes in the North Atlantic ecosystem brought about by regime change in the 1990s (deYoung *et al.* 2004; MERCINA Working Group 2012) and its

possible consequences for prey consumption for Atlantic salmon condition and survival, we sought to examine the influence of exogenous (i.e., diet and environment) and endogenous (i.e., size) factors on the variation in lipid content of Atlantic salmon feeding at West Greenland. With regard to exogenous factors, we tested the hypotheses that dorsal muscle lipid reserves of Atlantic salmon at West Greenland would: [H1] vary positively with week of capture, a proxy for time spent feeding at West Greenland, [H2] vary negatively with sea surface temperature (SST) given known links between fish physiological performance and temperature; [H3] correlate positively with $\delta^{15}N$ (a proxy for trophic position; Vander Zanden *et al.* 1997) and [H4] $\delta^{13}C$ values (a proxy for inshore feeding; Hansen *et al.* 2012; Sherwood & Rose 2005), and [H5] be positively correlated with the proportion of prey item types found in the gut, i.e., that because of variations in prey energy densities lipid reserves would depend on prey selection. With respect to the endogenous factors known to control lipid accumulation in fish (Sheridan 1994), we tested the hypotheses that dorsal muscle lipid reserves of Atlantic salmon at West Greenland would be: [H6] positively correlated with fork-length, and [H7] negatively correlated with marine growth rate during the second summer at sea.

Materials and Methods

Sample Collection

Atlantic salmon were sampled as part of the North Atlantic Salmon Conservation Organization's (NASCO) Salmon at Sea (SALSEA) Greenland sampling program (ICES 2012). Fish were captured (2009-2011) at three West Greenland communities: Sisimiut, Nuuk, and Qaqortoq, separated by approximately 875 km of coast (Fig. 3.1). Intensive sampling occurred from August to October. Dorsal muscle fillets were immediately frozen at -20°C for stable

isotope and lipid analyses (Dixon *et al.* 2017). Stomachs were collected and preserved for standard gut content analysis (Renkawitz *et al.* 2015). The proportion of a prey species in the gut was determined by dividing the weight of a given prey species by the total stomach content weight (Crisp *et al.* 1978). Adipose fin clips were removed and preserved for genetic determination of continent of origin (King *et al.* 2001; Sheehan *et al.* 2010), and scales were collected for age and growth determination (Power 1987). Only data for one sea-winter (1SW) North American-origin fish are reported in this study.

Lipid Extraction, Stable Isotope Analysis, and Scale Growth Analysis

Lipids were extracted using the gravimetric method described in Folch *et al.* (1957). Briefly, duplicates of freeze-dried, ground muscle tissue was combined with 2:1 chloroform:methanol and 0.9% potassium chloride, homogenized, and centrifuged. The resulting supernatant was removed, and the process was repeated two additional times. The extracted solution was then dried under nitrogen gas and combined with 2mL of 2:1 chloroform:methanol from which two 100mL aliquots of solution were removed and then dried and weighed (See Fagan *et al.* (2011) and Dean *et al.* (in review) for additional extraction methodological details). All values were reported as percent of wet weight (referred to hereafter as percent lipid).

Sea surface temperature (SST) data were obtained from NOAA's OI SST V2 database (NOAA Earth System Research Laboratory, Physical Sciences Division; https://www.esrl.noaa.gov/psd/; download date: Dec. 13, 2017) and are expressed here as the average SST during the three weeks prior to the date of fish capture. SST data were averaged

over a 0.5 degree radius of over ocean grids proximate to the community of capture to account for possible within ocean movements prior to capture.

For stable isotope analyses, all dorsal muscle tissue samples were dried or freeze-dried before being ground (Dixon *et al.* 2017). The ground tissue was then analyzed at the University of Waterloo Environmental Isotope Laboratory following standardized methods as described in Dixon *et al.* (2017). Reported analytical precision was ±0.2‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$. Precision was established with reference to the International Atomic Energy Agency standards CH6 (carbon) and N1 and N2 (nitrogen). All isotope values are reported with respect to the international reference values for Vienna Peedee Belemnite limestone, VPDB, for $\delta^{13}C$ (Craig 1957) and atmospheric nitrogen for $\delta^{15}N$ (Mariotti 1983).

Growth was assessed using images of impressed scales taken with an Olympus DP70 or DP72 camera using the software packages DPManager and DPController or Cellsens Entry (Olympus Corp., Tokyo, Japan). One scale image from each individual fish was analyzed using the software package ImagePro Plus 7 or ImagePro Express 6.3 (Media Cybernetics, Inc., Rockland, MD). Measurements were taken from the focus to the anterior edge of the scale (Haas-Castro *et al.* 2006). Size at the first marine annulus was determined using the Fraser-Lee back-calculation method (Lee 1920). Growth rate was then calculated using the formula:

$$\frac{\ln[L_c] - \ln[L_i]}{T_c - T_i}$$

where L_c is length at capture, L_i is length at the i^{th} annulus, T_c is time at capture (number of months from February), and T_i is time at the i^{th} annulus (always denoted as 1).

Prey items for stable isotope analysis were collected from the gut contents of 249 frozen stomachs obtained from the three years of sampling. Prey samples were chosen for stable isotope analysis if the sample was obtained from the foregut appeared freshly consumed, and the

epidermal layer showed no signs of digestion. Such samples are considered suitable for stable isotope analysis if washed in distilled water to remove digestive fragments and epithelial cells from the lining of the digestive tract (Grey *et al.* 2002; Guelinckx *et al*. 2008). Fish muscle tissue from prey samples were prepared for stable isotope analysis as described for Atlantic salmon. For boreoatlantic armhook squid, mantle tissue was subsampled, freeze-dried, and ground. All crustacean prey samples were acidified to remove inorganic carbon (Stasko *et al.* 2016), freeze-dried, and ground whole (Søreide *et al.* 2006). All ground tissue was then submitted to the University of Waterloo Environmental Isotope Laboratory for further analysis as previously described.

Statistical Analysis

All statistical tests were performed using RStudio version 1.0.143 (RStudio Inc., Boston, MA). Percent lipid, $\delta^{15}N$, $\delta^{13}C$, and fork length values beyond 1.5 times the interquartile range ± the median were considered as outliers and removed (n = 38). All percent lipid values were logit-transformed for statistical analysis to ensure conformance to normality assumptions (Sokal & Rohlf 1995; Warton & Hui 2011). The significance of exogenous (e.g., temperature, stable isotope measures) and endogenous (e.g. length, growth) factors expected to correlate with percent lipids were assessed using Pearson correlation analysis [H1-H4, H6, H7] and/or a quadratic regression model [H2]. The relative importance of identified significant factors was then assessed in a linear mixed model that included consideration of all identified significant correlative factors and significant two-way interactions (also as determined through correlation analysis). Backward selection and AIC were used to choose the final model [H1-H4, H6, H7]. The relative importance of all significant terms in the final AIC model was determined using

standardized beta coefficients (Zar 2010) and Akaike importance weights calculated as the sum of Akaike weights for each of the estimated models containing the significant term (Burnham and Anderson 2002).

The prey composition in consumption hypothesis [H5] was assessed using all possible combinations (n=15) of the four main prey items (i.e. capelin, boreoatlantic armhook squid, sand lance, and *Themisto* spp.) in the gut contents. The proportional weight of a given combination of prey items in the gut was summed and regressed against percent lipid data to determine significance. All residuals from all regressions were checked for normality and homogeneity of variance (Zar 2010).

Results

Lipids were extracted from 893 North American 1SW Atlantic salmon captured during the 2009-2011 sampling period at West Greenland. Average percent lipids varied among years and communities, but ranged from 9.15 ± 2.84% (mean ± standard deviation) at Nuuk in 2010 to 18.03 ± 5.54% at Sisimiut in 2009. Mean $\delta^{13}C$ values for North American 1SW Atlantic salmon varied from -21.02 ± 0.75 at Sisimiut in 2010 to -22.08 ± 0.98 at Qaqortoq in 2010, while mean $\delta^{15}N$ values varied from 11.25 ± 0.58 at Qaqortoq in 2010 to 11.87 ± 0.49 at Sisimiut in 2011.

Lipids were positively correlated with week of capture (H1: $p < 0.001$; Table 3.1), and were best described by a quadratic regression model ($r^2 = 0.032$, $p < 0.001$; Fig. 3.2), with mean percent lipids content reaching a maximum at week 40 (approximately October 1st). Lipids were negatively correlated with SST (H2: $p < 0.001$), positively correlated with $\delta^{15}N$ (H3: $p < 0.001$), and, in contrast to expectations, negatively correlated with $\delta^{13}C$ (H4: $p < 0.001$). There was no support for the prey composition consumption hypothesis [H5], with the lowest observed p for

any of the tested combinations of key prey items equalling 0.158 (Fig. 3.3). While fork length was not significantly correlated with lipid content (H6: p=0.377), growth rate was negatively correlated with lipid content (H7: p<0.001). Among the tested exogenous and endogenous factors, significant interactions were found between week of capture and length (r^2 = 0.116, p = 0.001), between $\delta^{13}C$ and $\delta^{15}N$ (r^2 = 0.116, p < 0.001), between SST and $\delta^{15}N$ (r^2 = -0.278, p < 0.001), between SST and growth rate (r^2 = 0.141, p < 0.001), between length and $\delta^{13}C$ ((r^2 = -0.140, p < 0.001), and between length and growth rate (r^2 = 0.104, p = 0.003).

When the significant SST, $\delta^{15}N$, $\delta^{13}C$, growth rate, and significant two way interaction correlates were considered in a multiple regression model using backwards stepwise-AIC, the best model with an Akaike weight of 0.366 incorporated $\delta^{13}C$ and the interaction terms: week of capture*$\delta^{15}N$ and SST*growth rate to explain variation in the lipid content of West Greenland Atlantic salmon (r^2 = 0.108, AIC_c = -225.65, Table 3.2; Fig. 3.4). The interaction between SST and growth rate was most important in the model followed by $\delta^{13}C$ and interaction of week of capture and $\delta^{15}N$ (Table 3.3).

Discussion

Data presented here for summer feeding North American 1SW Atlantic salmon at West Greenland indicate correlations between exogenous and endogenous determinants of muscle lipid concentrations including: week, SST, $\delta^{13}C$, $\delta^{15}N$, and growth rate during the second summer at sea. Interactions among primary determinants were also important as evidenced by the AIC determined best model for explaining lipid. Percent lipids did not correlate with the proportion of any single prey item or proportional combinations of prey items found in the gut contents.

Lipid reserves increased temporally with week of capture until standard week 40, suggesting that feeding conditions and associated opportunities for lipid accumulation likely remain favorable along the West Greenland coast until the end of September. Changes in prey abundance, quality, or spatial distribution linked to changes in SST at the end of September may be associated with the declines in percent lipid observed after week 40. The weakness of the correlation between week of capture and percent lipids may relate to differences in arrival timing at West Greenland, as fish may be arriving from over-wintering areas in the Labrador Sea at varying times through the summer (Dadswell *et al.* 2010). Variations among years in the origin of sampled fish (e.g., Reddin et al. 2012) may have further contributed to the weakness of observed correlations. Additionally, as lipids are allocated to the muscle tissue as secondary storage and mobilized more readily than mesenteric lipids (Sheridan 1994), the weakness of the correlation may be confounded by inter-individual differences in the rates of lipid assimilation from the muscle tissue or non-homogenous distribution of lipids within muscle tissue (Katikou *et al.* 2001).

Results here indicate ocean temperature appear to be impacting Atlantic salmon lipid reserves. Changes in SST have been found to have conflicting impacts on Atlantic salmon that depend on continent of origin and age, with high ocean temperatures being linked to increased survival in Atlantic salmon off the coast of Norway (Friedland *et al.* 2000), increased growth in Canadian Atlantic salmon post-smolts, and decreased growth in Scottish post-smolts (Friedland *et al.* 2005). Given that growth and lipid storage are negatively related (Jonsson & Jonsson 2011) as a result of increases in lipid metabolism as fish grow (e.g., Elliott 1994), a priori expectations were for a strong relationship. At constant ration, increases in SST should increase the metabolic costs of fish residing off the West Greenland coast (e.g., Elliott 1994, Jonsson & Jonsson 2011).

The weakness of the observed correlation between SST and lipids may arise because of the ability of individuals to compensate for changes in SST through utilization of differing water depths, e.g., via behavioural thermoregulation (Minke-Martin *et al.* 2015) or via differences in arrival timing to feed on West Greenland coast. Use of spatially averaged SST data assumes Atlantic salmon have been resident in the capture area for a minimum of three weeks, whereas fish may have experienced anything in the range (4-10^{o} C) of temperatures they are known to inhabit in the marine environment (e.g., Minke-Martin *et al.* 2015). Furthermore, the impacts of temperature on lipid accumulation dynamics will depend critically on ration, with the scope for growth at a given temperature varying with ration (Elliott 1994). Nevertheless, the detection of significant correlation between lipids and SST suggests climate-driven increases in SST will remain a concern for Atlantic salmon. Mean lipid reserves are likely to decrease as temperatures rise as a result of increasing metabolic maintenance costs and reduced abilities to trade-off between growth and lipid accumulation, suggesting continued or intensified decreases in marine survival and productivity associated with warming seas (Elliot 1994; Handeland *et al.* 2008; Jonsson & Jonsson 2011).

The positive correlation between percent lipids and δ^{15}N indicates that fish consuming higher trophic level prey tend to have better muscle lipid levels. Renkawitz *et al.* (2015) suggested that feeding on larger, higher trophic level prey was more energetically beneficial as a result of less energy being expended for prey capture and digestion. Prey noted to have higher δ^{15}N values, such as capelin and boreoatlantic armhook squid (Linnebjerg *et al.* 2016) were also noted to have higher energy densities (Elliott & Gaston 2008; Hedeholm *et al.* 2011). As an opportunistic, generalist predator (Lear 1972; Jacobsen & Hansen 2001; Dixon *et al.* 2017), the ability of Atlantic salmon to switch between prey types appears to enable them to compensate for

declines in a given prey, e.g. capelin, while at the same time maintaining lipid levels. For example, locational differences in prey consumption reported by Dixon *et al*. (2017), i.e. dominance of capelin or boreoatlantic armhook squid appear to have had little impact on lipid values as reported by Dean *et al*. (in review).

The importance of interactions between week of capture and $\delta^{15}N$ suggests that the timing of arrival to West Greenland is likely important for forage success and energy acquisition. Danielsen *et al.* (2016) noted that the lipid content of the northern sand lance, *Ammodytes dubius*, peaked in August for mature fish and September for juveniles. Matching time of arrival with the availability of high quality prey is, therefore, likely to play a key role in the determination of individual lipid reserves, with lipid reserves having been used to identify match-mismatch situations in the larval sprat (*Sprattus sprattus*) in the Baltic Sea (Peters *et al.* 2015). While a large literature exists (e.g., Peck *et al.* 2012) that examines the implications of match-mismatch dynamics of fish and their prey for larval development, growth and eventual survival, a more subtle match-mismatch dynamic that extends beyond critical juvenile periods (e.g., Bollens *et al.* 1992) may be similarly affecting the growth and conditioning of adult phases of the Atlantic salmon life-history.

Given that lipids correlated negatively with $\delta^{13}C$, it appears that fish spending more time feeding offshore accumulate the greatest quantities of lipids. While nearshore areas may be more biologically productive, metabolic costs are higher given increased SST (Handeland *et al.* 2008; Arendt *et al.* 2010; Ribergaard 2011, 2012) and the increased energy available from more productive nearshore areas does not appear to offset the increase in metabolic costs of nearshore residency.

Energetic trade-offs between the accumulation of lipids and growth (Jonsson & Jonsson 2011) are consistent with the negative correlation between growth rate and percent lipids found in this study. Lipids, however, are routed to the liver and mesenteric fat in addition to muscle tissue, and the weak correlation between growth rate and percent lipids in the muscle tissue may reflect preferential routing lipids to mesenteric fat and in liver tissue (Sheridan 1994).

Additionally, Fraser *et al.* (2007) noted that Atlantic salmon experienced compensatory growth at different rates based on migration distance, with fish migrating the furthest growing the fastest. Thus fish making the longest migrations to West Greenland would likely accumulate fewer lipids. Given that the Atlantic salmon in this study represent populations of salmon from differing regions of North America with varying migration distances (Bradbury *et al.* 2016), it is also possible that compensatory growth is occurring at different times and to different degrees in West Greenland salmon. Any relationship between growth during the second summer at sea and lipid accumulation would be further weakened by differences in compensatory growth as growth would vary based on migration distance. The significance of a negative relationship between percent lipids and the interaction between growth and SST indicates that as temperatures continue to increase, Atlantic salmon lipid reserves will likely decrease.

Lack of significant correlations between percent lipids and the proportion of prey in the gut contents for the four major prey species (Renkawitz *et al.* 2015) suggests that short-term snap shots of feeding success (i.e. gut contents analysis) do not predict long term indicators of fish condition (i.e. lipid stores) which are likely to be linked to eventual survival and spawning success (Herbinger & Friars 1991; Todd *et al.* 2008; Jonsson & Jonsson 2011). Diet was noted to vary greatly with time and space over the period of feeding at West Greenland (Renkawitz *et al.* 2015; Dixon *et al.* 2017) likely given feeding strategy, local prey availability, and movement to

and around the West Greenland coast (Lear 1972; Jacobsen & Hansen 2001; Dadswell *et al.* 2010; Dixon *et al.* 2017). Therefore, limited nature of gut contents analysis as a measure of feeding behavior and long-term diet variability is likely responsible for the lack of observed correlation between prey proportion in the gut contents and lipids.

Forage success likely accounts for some of the observed variation in lipid content. Fish that are more successful foragers acquire more energy that can be used for lipid accumulation. As Kadri *et al.* (1996) observed variability in fish muscle lipid content with constant availability of food, it is likely that differences in foraging success would result in increased variability in muscle lipid content, further masking the relationship between exogenous and endogenous factors and muscle lipid levels. Although possibly biased by method of capture, measures of stomach fullness and diet composition recorded for the fish used in this study and the diet studies of Renkawitz *et al.* (2015) and Dixon *et al.* (2017) showed considerable variability. Among-individual differences in $\delta^{13}C$ (Dixon *et al.* 2017) further suggested among-individual differences in inshore and offshore feeding. Additionally, weak correlations in all instances may indicate that there is a lag between feeding and the assimilation of lipids to muscle tissue with lipids in the muscle tissue being considered a secondary location for lipid storage (Sheridan 1994). In addition, individual fish are likely exposed to disease, predators, and parasites at different rates given differences in movement (Dadswell *et al.* 2010). Thus, the increased stress response induced by resisting disease and parasites or the increased energy used to avoid predators may account for observed variability and the weakness in observed correlations (Houston *et al.* 1993; Jonsson & Jonsson 2011).

Conclusion

Data from this study provides the first assessment of the relative importance of endogenous and exogenous determinants of variation in dorsal muscle lipid content in summer-feeding 1SW North American origin Atlantic salmon captured at West Greenland. Differences in the timing of arrival at West Greenland are argued to explain the weak correlation between week of capture and percent lipids. Correlation between SST and percent lipids suggest that continued ocean warming will continue to have adverse effects on Atlantic salmon lipid storage and ultimately survival. Fish feeding offshore and on higher trophic level prey have higher lipid stores, which may predispose them to better survive the overwintering period and return migration to natal spawning rivers. Interactions between week of capture and $\delta^{15}N$ suggest that timing of arrival (match-mismatch) is critical for feeding success and lipid accumulation. Interactions between SST and growth rate suggest that as SST continues to increase, fish will be less capable of allocating energy to lipid accumulation and growth. Absent correlations between the proportion of prey in the gut and percent lipids suggest that in opportunistic feeders such as Atlantic salmon overall energy accumulation will have greater influence on lipid accumulation and survival than the selection of any single prey type. While data available for testing the influence of endogenous factors on lipid accumulation were not extensive, exogenous environmental factors appear to have the greatest impact on Atlantic salmon lipid accumulation. Data from this study also demonstrates the need for repeat measurements of lipid content and diet in individual fish with time and suggest that further research is needed to assess the assimilation dynamics of lipids in wild Atlantic salmon.

Table 3.1. Correlation coefficients for exogenous and endogenous factors used to assess factors explaining lipid content 1SW North American origin Atlantic salmon samples at West Greenland. The upper value represents the Pearson correlation coefficient while the lower value represents the p-value. Bolded values indicate significant correlations.

	Percent Lipids	Week of Capture	SST	Length	$\delta^{13}C$	$\delta^{15}N$	Growth Rate
Percent Lipids	1.000	**0.142**	**-0.214**	0.031	**-0.173**	**0.165**	**-0.142**
	-	**0.000**	**0.000**	0.377	**0.000**	**0.000**	**0.000**
Week of Capture		1.000	-0.041	**0.116**	**-0.123**	**0.258**	-0.021
		-	0.235	**0.001**	**0.000**	**0.000**	0.553
SST			1.000	-0.008	0.016	**-0.278**	**0.141**
			-	0.822	0.647	**0.000**	**0.000**
Length				1.000	**-0.140**	0.068	**0.104**
				-	**0.000**	0.051	**0.003**
$\delta^{13}C$					1.000	0.018	-0.006
					-	0.602	0.867
$\delta^{15}N$						1.000	-0.024
						-	0.493
Growth Rate							1.000
							-

Table 3.2. Summary of AIC model selection results for models explaining percent lipids in 1SW North American origin Atlantic salmon sampled at West Greenland. The best 5 models are presented.

Model Number	Model	K	LogLik	RSS	AICc	Δ	W_i
1	Percent Lipid = $\delta^{13}C$, SST*Growth rate, Week of capture*$\delta^{15}N$	5	117.86	36.30	-225.65	0.000	0.366
2	Percent Lipid = $\delta^{15}N$, SST*Growth rate, Week of capture*$\delta^{15}N$, Week of capture*$\delta^{13}C$	6	118.13	36.27	-224.17	1.480	0.175
3	Percent Lipid = $\delta^{13}C$, $\delta^{15}N$, SST*Growth rate, Week of capture*$\delta^{13}C$	6	118.01	36.29	-223.92	1.730	0.154
4	Percent Lipid = $\delta^{13}C$, $\delta^{15}N$, Week of capture, SST*Growth rate	6	118.03	36.28	-223.95	1.700	0.156
5	Percent Lipid = $\delta^{15}N$, Week of capture, SST*Growth rate, Week of capture*$\delta^{13}C$	6	117.98	36.29	-223.85	1.800	0.149

Table 3.3. Beta coefficients and Akaike importance weights for terms in the final model explaining percent lipids in 1SW North American origin Atlantic salmon sampled at West Greenland as selected by backwards stepwise AIC. Standard error represents the standard error for the reported beta coefficients.

AIC Model Term	Beta	Standard Error	Akaike Importance Weight
SST*Growth Rate	-0.217	0.033	0.965
$\delta^{13}C$	-0.16	0.033	0.627
Week of capture*$\delta^{15}N$	0.146	0.033	0.461

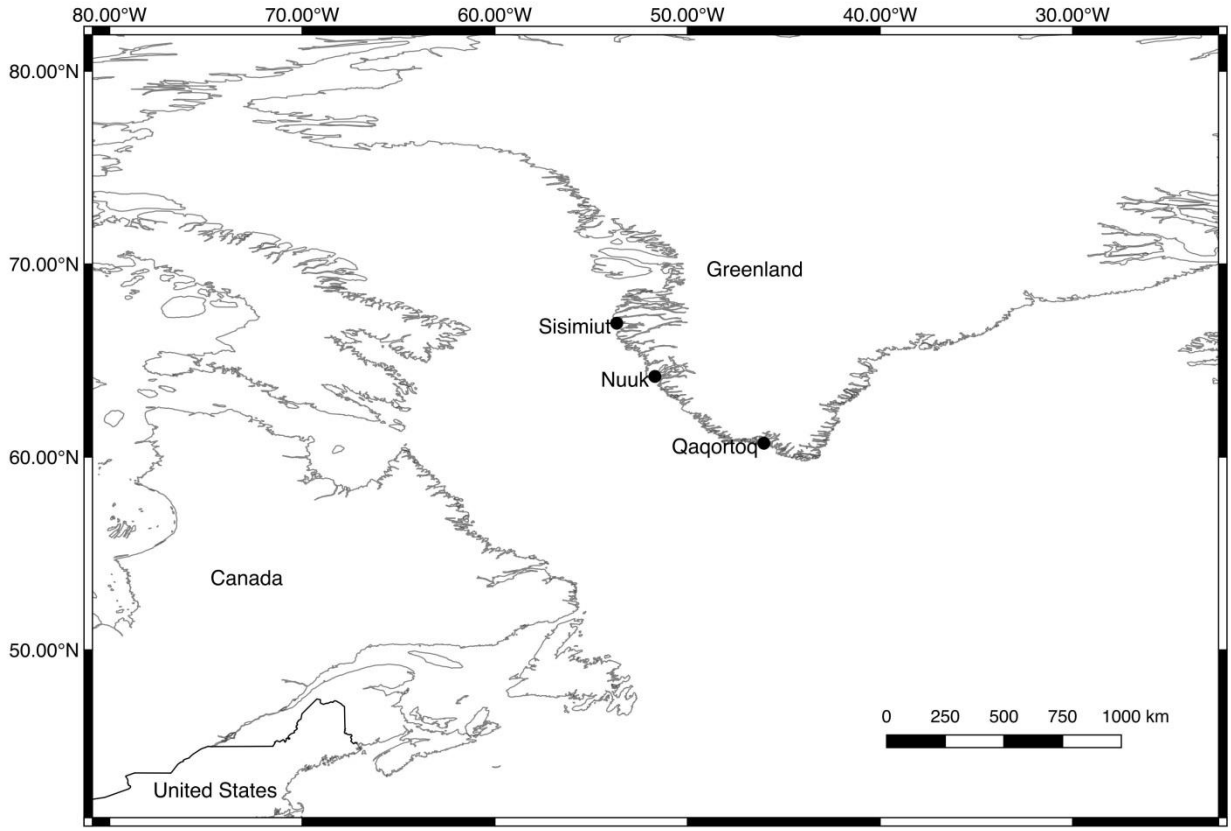

Figure 3.1. 1SW North American origin Atlantic salmon sampling locations along the West Greenland coast from 2009 to 2011.

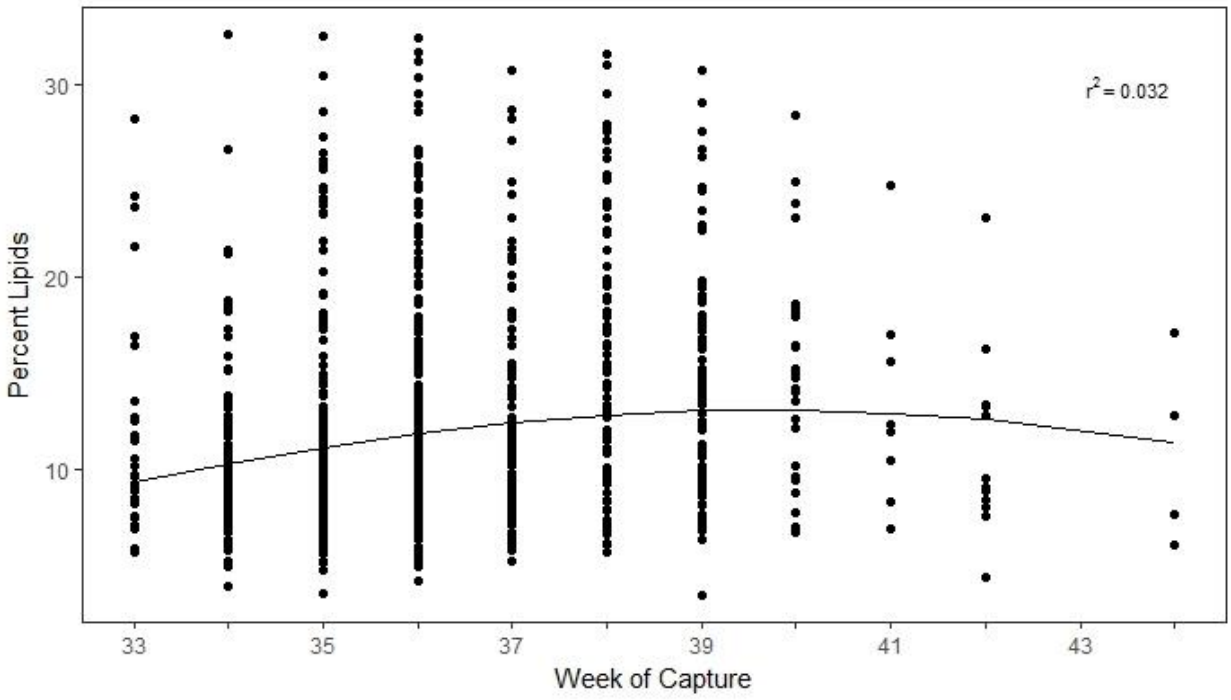

Figure 3.2. Correlation between percent lipids and week of capture for 1SW North American origin Atlantic salmon sampled at West Greenland. Week of capture reflects standard calendar week, where week 33 begins on August 13th and week 44 ends on October 28th.

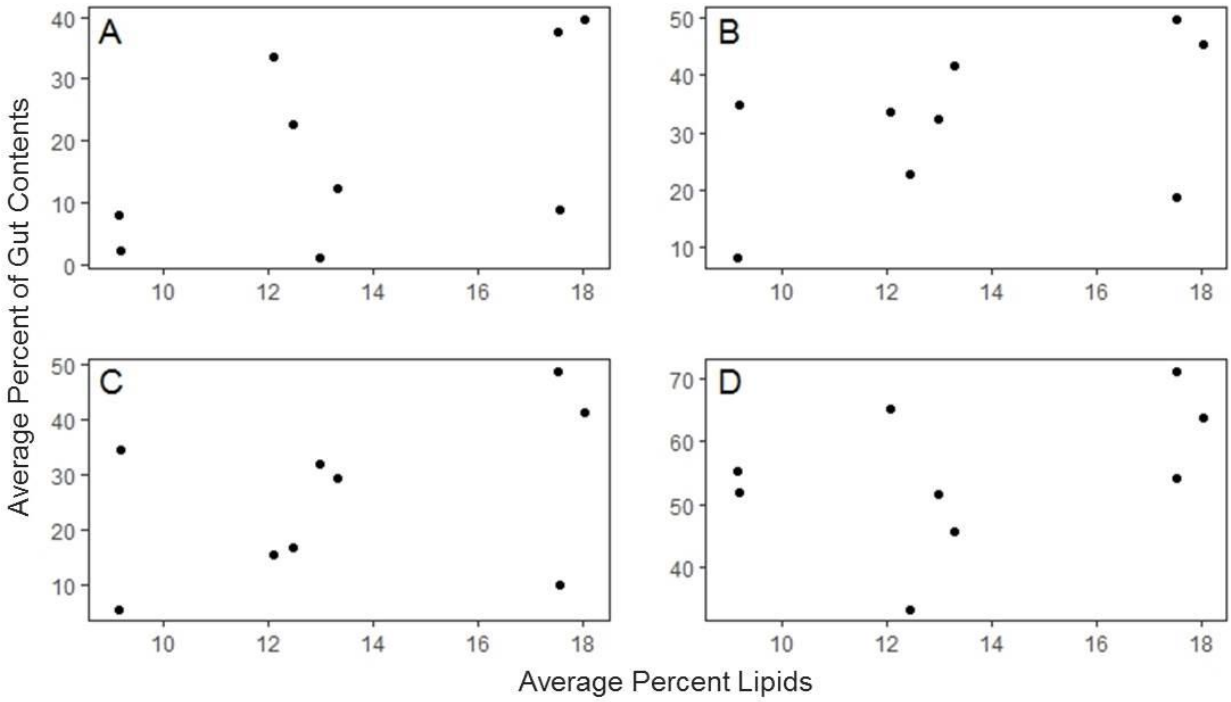

Figure 3.3. Correlation between average percent lipid and the average proportion of prey found in the gut contents 1SW North American origin Atlantic salmon captured at West Greenland. The four of n=15 regressions of combinations of prey with the lowest p-values are plotted: sand lance and boreoatlantic armhook squid (average 18.45% of gut contents: p = 0.158; A), sand lance, boreoatlantic armhook squid, and *Themisto* spp. (average 31.98% of gut contents: p = 0.177; B), *Themisto* spp. and boreoatlantic armhook squid (average 26.00% of gut contents: p = 0.285; C), and capelin, sand lance, and boreoatlantic armhook squid (average 54.74% of gut contents: p = 0.323; D). Points reflect averages for each community for each year of sampling.

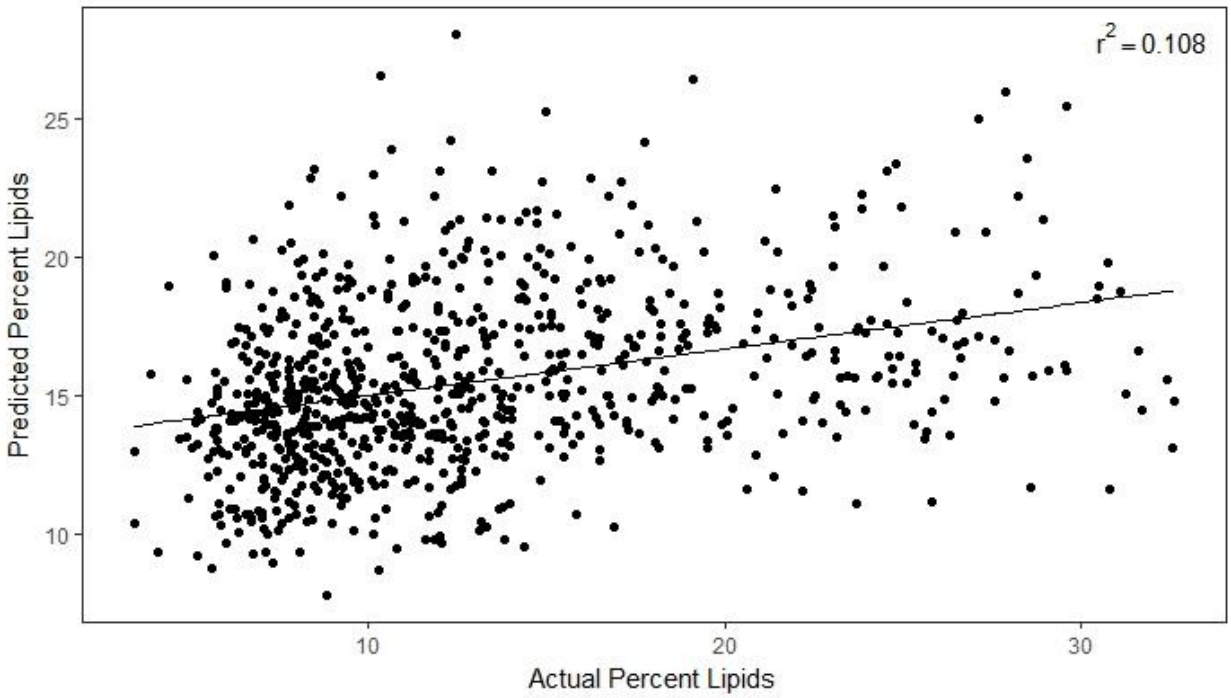

Figure 3.4. Correlation between model-predicted percent lipids and actual percent lipids for 1SW North American origin Atlantic salmon sampled at West Greenland. Predicted values represent those predicted by the best AIC selected model using the interactions between SST and growth rate and week of capture and $\delta^{15}N$, and $\delta^{13}C$ data.

Chapter 4: General Conclusions

Summary of findings

Chapter 2 quantified variation in the lipid content of Atlantic salmon captured at West Greenland across different sea ages, continents of origin, regions of origin, and latitudes. Although differences in prey consumption were not noted by age class in an associated feeding study of the same individuals used in this study (Renkawitz *et al.* 2015), the correlation between increasing size (i.e., one sea-winter versus multi-sea-winter) and lipid content noted here may have been facilitated by increases in mouth gape allowing for the consumption of larger prey items (Thorpe 1986; Kadri *et al.* 1996; Jacobsen & Hansen 2001). Lack of differences in percent lipids by continent and region of origin further suggest prey selection within common feeding areas and/or localized variation in marine environmental conditions are important for explaining among individual differences in lipid storage. The ability to link differences in lipid content to larger scale factors, such as latitude or region of origin, may be confounded by differences in individual fish arrival timing along the coast (Rikardsen *et al.* 2006; Dadswell *et al.* 2010; Dixon *et al.* 2017).

Weak relationships between percent lipids, fish condition, and weight may also have been masked by differences in maturation and lipid allocation among individual fish and highlight the need to include estimates of mesenteric fat, gonads, and liver tissue samples in lipid studies (Simpson 1992; Sheridan 1994). This chapter suggests that prey type may be an important determinant of lipid accumulation given observed differences in lipid content by community, with increased lipid content in fish captured at Sisimiut where larger capelin and a higher

abundance of boreoatlantic armhook squid has been noted (Hedeholm *et al.* 2010; Renkawitz *et al.* 2015; Dixon *et al.* 2017).

Chapter 3 assessed correlations between the lipid content of North American-origin 1SW Atlantic salmon at West Greenland and residency timing, environment, diet, and fish-specific characteristics. Percent lipids showed a weak nonlinear relationship with week of capture, increasing with week of capture until week 40 (October 1st) and thereafter declining. The nature of the relationship suggests that changes in prey availability and/or condition and/or environmental conditions at the end of September result in a decline in lipid accumulation beginning in October. Percent lipids decreased with increasing sea surface temperature (SST), likely as a result of the increases in metabolic demand associated with increases in temperature (Handeland *et al.* 2008; Jonsson & Jonsson 2011). The link to metabolic demand suggests that continued increases in ocean temperature due to climate warming may exacerbate declines in lipid stores and condition already noted in some European Atlantic salmon stocks (Todd *et al.* 2008). Percent lipids correlated positively with $\delta^{15}N$, indicated that feeding at higher trophic levels is beneficial for increased lipid accumulation. Additionally, percent lipids were negatively correlated with $\delta^{13}C$, suggesting that fish feeding offshore accumulate the largest lipid reserves. While nearshore areas are thought to be more productive, the increased energy available does not appear to offset the increased metabolic cost of being in warmer inshore waters (Arendt *et al.* 2010; Ribergaard 2011, 2012). Therefore, cooler offshore areas, while less productive maybe more favorable to lipid accumulation. The observed negative correlation between percent lipids and growth rate during the second summer at sea, reinforces the idea that there are critical trade-offs between growth and lipid accumulation for adult Atlantic salmon (Jonsson & Jonsson 2011). Overall, percent lipids were found to be best explained by a multivariate model that included

consideration of interactions between $\delta^{15}N$ and week of capture, $\delta^{13}C$, and interactions between SST and growth rate during the second summer at sea. The model interactions suggest the importance of arrival timing relative to forage success and the impact of SST on energetic trade-offs between growth and lipid storage. The insignificance of observed correlations between percent lipids and proportion of prey in the gut contents suggests that overall energy accumulation is more important for lipid storage than the type of prey consumed. Finally, the lack of correlation of lipids with gut contents suggests that they per se do not reflect long-term trends in the consequences of prey selection for fish condition and its associated vital rates correlates (e.g., survival probability, fecundity).

Study significance

To the author's knowledge, this thesis is the first study to examine the lipid reserves of summer-feeding Atlantic salmon at West Greenland. As multi-sea-winter salmon have continued to decline in abundance (ICES 2016), the need for research exploring potential causal mechanisms for the decline of Atlantic salmon in the marine environment has become increasingly important. This study helps address possible causal mechanisms by linking environmental and biological factors to lipid accumulation, with lipids being critical for maturation, survival during migration, and reproduction (Thorpe 1986; Jonsson & Jonsson 2011).

Chapter 2 addressed the influence of age, continent of origin, and region of origin on lipid reserves and made linkages between weight, fish condition, and lipid content that stressed the importance of understanding fish movement around the West Greenland coast and the need to incorporate measures of whole fish lipids into future analyses. Chapter 3 filled gaps in

knowledge about the possible mechanisms driving observed variation in lipid content, suggesting that overall energy acquisition is more important than feeding on a particular prey source and that climate change will likely contribute to observed further declines in Atlantic salmon condition. Chapter 3 also suggested that gut contents analysis and single measures of lipid content are not sufficient to accurately predict trends in the diet and lipid accumulation over the life cycle of the fish.

Evidence presented in both chapters suggests that energetic trade-offs favor the allocation of energy towards growth rather than lipid accumulation. Climate change will likely continue to exacerbate this effect, as interactions between temperature and growth rate correlated negatively with percent lipids (Chapter 3). This suggests that Atlantic salmon that have fed at West Greenland may not meet the energetic requirements for maturation (Thorpe *et al.* 1998) and migration. Thus fish deciding to make the return migration to reproduce may have less energy to utilize during migration (Keissling *et al.* 2004), potentially resulting in increased mortality during migration, or less energy available for reproduction as noted in Todd *et al.* (2008). Together, these effects may account for the observed declines in MSW Atlantic salmon (ICES 2016).

Future work

While this thesis has helped to increase scientific knowledge of the marine life history of Atlantic salmon by assessing the lipid content in West Greenland marine-feeding fish, there is much more work that is needed to fully understand the reasons for the continued decline of Atlantic salmon stocks.

As mentioned in Chapter 2 and Chapter 3, lipids are allocated to the liver and mesenteric fat in addition to muscle tissue, and mesenteric fat is considered the primary depot for lipids in many species of fish (Sheridan 1994). In addition, it is possible that individuals allocate lipids to these different regions to different degrees and at differing times. Therefore, to more accurately account for lipid variation, future studies should seek to assess whole body lipids through the homogenization of the entire fish and assess differences in lipid content between muscle tissue, mesenteric fat, and the liver.

It is important that future studies seek to better quantify movement along the West Greenland coast. Chapter 2 noted that differences in arrival timing at different areas of the West Greenland coast may impact the assessment of latitudinal differences in lipid content. Currently, the knowledge of Atlantic salmon movement at West Greenland and during the second year at sea as a whole is not well understood (Dadswell *et al.* 2010). Understanding the movement of fish along the West Greenland coast will allow for greater quantification of regional differences with time. Movement information would increase abilities to assess the impacts of local environmental factors on lipid accumulation. In addition, knowledge of movement and/or local residency would allow for better quantification of thermal habitat use with time, thereby improving the ability to link condition and/or lipid indices with experienced SST.

Chapter 3 suggested that gut contents analyses and single measures of lipid content are not effective in accurately predicting long-term diet and lipid reserves. Future work, therefore, should seek to use lipid and gut contents analyses to assess changes in diet and lipid accumulation with time in individual fish with repeat measurements. Although technically challenging, repeat measurements of lipid content and diet would improve understanding of relationships between lipid accumulation, ocean temperature, growth, maturation, feeding

behavior, forage success, and time. Such data would improve the ability to assess how rapidly lipid reserves change with changing environmental factors, thereby helping to better predict fitness for migration and reproduction.

While much emphasis has been put on sampling Atlantic salmon at West Greenland given its importance as a summer feeding area for Atlantic salmon, it is likely that other areas and times during the marine residency of Atlantic salmon are as, or are more, critical for the accumulation of lipids. Sampling at different areas, such as the overwintering area in the Labrador Sea, and in different seasons would allow for increased understanding of temporal and spatial changes in lipid content with time by providing greater insights into the environmental factors, regions, or seasons that play a critical role in lipid accumulation.

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